EARLY LIFE-HISTORY OF *MELAMPUS* AND THE SIGNIFICANCE OF SEMILUNAR SYNCHRONY [1]

W. D. RUSSELL-HUNTER, MARTYN L. APLEY AND R. DOUGLAS HUNTER[2]

Department of Biology, Syracuse University, Syracuse, New York 13210; Department of Biology, Brooklyn College, Brooklyn, New York 11210; and the Marine Biological Laboratory, Woods Hole, Massachusetts 02543

Living in the higher levels of salt marshes, pulmonate snails of the genus *Melampus* are truly "amphibious." In their respiration, they are land snails breathing air through pneumostome and lung. In their reproduction, they are primitive marine snails spawning large numbers of small eggs which yield planktonic veliger larvae on hatching. As adumbrated in earlier publications on the biology of *Melampus bidentatus* (Russell Hunter and Apley, 1966; Apley, Russell-Hunter and Avolizi, 1967; Apley, 1970; Russell-Hunter, Apley and Hunter, 1970), there is a significant temporal "fitting" of the reproductive events including egg-laying and hatching to the two-week periodicity of spring tides. The sequence and "control" of these events and, in addition, of those of veliger settlement, metamorphosis and spat growth have now been examined in greater detail and the more significant observations are reported in this paper. Minor parts of this account are derived from observations in the summers of 1964 and 1966, but most of the detailed observations on sequential "timing," the experiments on "controls" and the observations on laval and spat growth, settlement and metamorphosis were made and integrated during the summer of 1970 at Woods Hole.

Melampus bidentatus Say is a species placed in the family Ellobiidae of the subclass Basommatophora, which group is generally regarded as encompassing the most primitive living "lung-snails" of the class Pulmonata. As in all pulmonates, the molluscan mantle-cavity has been modified to a gill-less vascularized lung, and ellobiids are functionally "air-breathers." However, certain anatomical features, including many in the nervous, excretory and reproductive systems of ellobiids, remain considerably less specialized than those in more typical Pulmonata (Morton, 1955a, 1955b), and the Ellobiidae retain a number of features more diagnostic of archetypic marine gastropods. In the life-cycles of certain ellobiid species, including *Melampus*, a free-swimming veliger larva is retained, as in *no* other pulmonates. It should be remembered that molluscs are largely marine. Only certain gastropods (the class Pulmonata, and—particularly in the tropics—certain genera in four prosobranch superfamilies) are found on land, and they probably number less than twenty per cent of molluscan species (Thiele, 1931; Russell Hunter, 1964; Boss, 1971).

[2] Present address: Department of Biology, Oakland University, Rochester, Michigan 48063.

As in such forms as the "primitive" vertebrate lung-fishes, considerable significance attaches to any observations on the structural and functional adaptations shown by amphibious snails such as ellobiids. For *Melampus bidentatus*, this amphibious animal living in the highest tidal zone of salt marshes, we can now describe an elaborate succession of temporal adjustments of reproductive development and early growth which corresponds to the pattern of spring tide submergence. The difficulties of rapid larval and spat growth from a relatively small "primitive" egg (and therefore from a relatively small veliger at hatching) to a miniature version of the adult snail capable of surviving its first winter in the upper levels of the salt marsh have also been investigated. The rapidly changing growth patterns involve a major shift in shell growth (or a metamorphosis) occurring after settlement. In discussing these shell growth changes, we are able to present an unusually complete picture of development in a case of "heterostrophy" of larval and adult shell.

Such observations on amphibious forms like *Melampus*, and more particularly data on their physiological ecology, can have two kinds of significance. First, at the level of mechanistic physiology, they help establish our concepts of how such an "unlikely" animal machine as one built on the basic molluscan plan—involving elaborate ciliary, mucous, and hydraulic mechanisms of great efficiency in an aquatic environment—can maintain itself on land. Secondly, it is possible that hypotheses on the evolution of the major land stocks can be modified as a result of physiological and ecological investigations of amphibious stocks. No one would claim that contemporary ellobiids were descended unchanged from the stocks ancestral to the present 8000 species of efficiently terrestrial Pulmonata, but they undoubtedly face the same physiological problems and live in the same variable habitats as did the actual Jurassic ancestors of these most successful of land snails.

Observations and Results

1. Eggs and egg-laying

The egg-masses are gelatinous and have no capsules or protective coatings such as are found in the majority of pulmonate snails. On laying, they are usually irregular hemispheres of 1–2 mm diameter, approximately 0.5 mm thick in the center. Each egg-mass is made up of from 539 to 1240 eggs (mean = 850 for 42 egg-masses counted) which are deposited in a single continuous strand consisting of evenly-spaced eggs (each 170 μ long, see Fig. 1A) enclosed in two tubular gelatinous layers.

The inner thicker material, concentrated primarily around the eggs but continuous within the egg-strand, can be referred to as the spherical envelope and is surrounded by the outer more tubular layer termed the string membrane (Fig. 1A). There is no direct evidence of the specific origins of these elements in different parts of the female genital tract, but it is tempting to suggest that the spherical envelope, which must be laid down first, is secreted by the albumen gland along with the egg-shell itself, and that the string membrane has its origin in the mucous gland. As described by Apley (1970), the ova in *Melampus* almost certainly traverse a channel running through the mucous gland prior to egg-laying. It would be misleading to attempt to homologize these two layers in
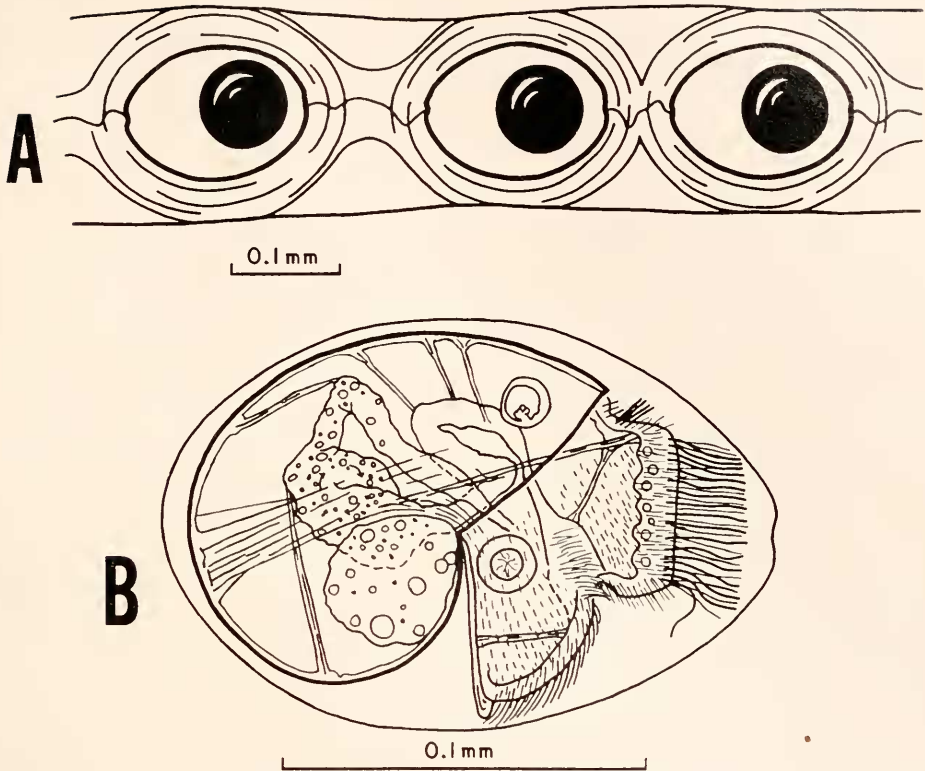
FIGURE 1. Eggs of *Melampus bidentatus:* (A) part of an egg-strand from a newly laid egg-mass, showing the two tubular gelatinous layers surrounding the eggs; and (B) an egg after 11–12 days of development containing a well-differentiated and active veliger larva.

Melampus with the detailed nomenclature set up for the higher limnic pulmonates by Bondesen (1950), but it is worth noting that no pedal glands are involved in this egg-mass secretion. Among the higher limnic Basommatophora, "external" secretions from the foot are important as the origin of the tough outermost coatings of the egg-capsules in such forms as ancylid limpets (Russell-Hunter, unpublished). The tubular string membrane in *Melampus* appears to form the general jelly matrix of the egg-masses as they "age" during the first days after laying, and is usually indistinguishable by the time of hatching. The spherical envelope, although continuous in the egg-strand, is concentrated primarily around the egg-shells themselves, appearing as optically distinct fine concentric bands in life, with lesser amounts in each "neck" between eggs. Within this, and connecting each egg with the preceding egg is a fine string-like structure, termed the allochalazal strand, which is probably continuous with the outer portion of the egg-shell. Attached at the points of indentation in the ends of the egg-shell (Figs. 1A and B), the allochalazal strand has been found to be mechanically important in the process of hatching which is described in the following section.

The organic content of the egg-mass layers (excluding the eggs themselves) is relatively low and it is obvious that free water is taken up at oviposition. Egg-

masses are never laid in totally dry conditions but, in contrast, adult snails are never submerged when egg-laying. The ecological and behavioral implications of this have been outlined before (Apley, 1970; Russell-Hunter *et al.*, 1970) and will be discussed in more detail below. Some biomass values can be given for the individual eggs. Mean dry weight is 354 ng (mean for 18 batches) and an average wet weight would be nearly 13 times greater at 4.72 μg (mean of 10 batches totalling 8,909 eggs). Wet weights of eggs are much influenced by any adherent envelope and membrane material from the mass, and attempts to standardize wet weights of entire egg-masses failed. In fact, a series of experiments with closed dishes at different relative humidities, showed that entire early egg-masses behaved like hygrometers in their relatively rapid weight responses. Similarly, submerging early egg-masses in waters of salinity range 25%–100% SW produced weight changes indicating that the masses were behaving like near-perfect osmometers. Both in the field, and in laboratory cultures, the egg-masses remain stiff turgid hemispheres for only about 3–4 days out of the 12–13 of development, and become more flaccid and irregular for the later two-thirds of the time until hatching. Total organic carbon content (determined by a wet oxidation technique: Russell-Hunter, Meadows, Apley and Burky, 1968) gave mean values of 109 ng carbon per egg (mean of 13 determinations totalling approximately 171 mg wet weight of eggs). Total nitrogen content (determined using a modified micro-Dumas technique on a Coleman semi-automatic nitrogen analyzer) was equivalent to 23.9 ng nitrogen per egg (mean of 9 determinations totalling approximately 14,000 eggs). In bioenergetic terms, these are remarkably small eggs for pulmonate snails. Comparable mean values for individual eggs in the freshwater limpet, *Lacvapex fuscus*, are 4.31 μg C and 1.35 μg N (McMahon, 1972) and for *Lymnaea palustris* 25 μg C and 3.61 μg N (Hunter, 1972). Actual egg production by *Melampus* was most carefully assessed in 1966, when the annual reproductive period encompassed three semilunar cycles of egg-laying (Apley *et al.*, 1967; Apley, 1970). Before reproduction began, groups of 10 to 58 snails were isolated in the laboratory in eight mass culture dishes and maintained in appropriate conditions of light and temperature on damp filterpaper. No attempt was made (or was needed, see below) to simulate tidal conditions. The observed groups totalling 244 mature snails laid 219 $\times$ 10⁴ eggs, then 345 $\times$ 10⁴ eggs, and finally 254 $\times$ 10⁴ eggs. Therefore, the overall fecundity totalled 818 $\times$ 10⁴ eggs, corresponding to an average of 33,150 eggs per snail per year, laid in an average of 39 egg-masses. Thus, despite the comparative minuteness of the individual eggs, the reproductive period represents a period of exceedingly high bioenergetic output for mature specimens of *Melampus*. Elsewhere (Apley *et al.*, 1967) we have computed that 87% of the non-respired assimilation (N-RA) is directed to egg-output during the reproductive period, corresponding to 46% of the total annual N-RA, or to 32% of the N-RA if spring pre-breeding growth rates were sustained throughout the year. This output per individual standard snail corresponds to 7.3 mg dry organic material annually. As is the case in the few invertebrate species yet studied bioenergetically, much of the reproductive output must be sustained by the concurrent food input being at a relatively high level for the species. However, in this work we noted a gonadal depletion during the three cycles of the reproductive period amounting to 78% of the initial organic carbon content and to over 91% of the total nitrogen corre-

sponding to a change of the C:N ratio of the gonad tissues from 6.3:1 to 15:1 (figures recalculated from data summarized in Apley *et al.,* 1967). This led to the hypothesis (Russell-Hunter, 1970) that egg-production was limited by the rate at which suitable organic nitrogenous materials could be made available to the gonad, in a fashion analogous to inorganic nitrogen as a limiting factor for the primary productivity of certain ecosystems.

As will be discussed, several aspects of survivorship during the early life-history of *Melampus* are related to this high numerical fecundity and the considerable bioenergetic output which it demands.

For obvious adaptive reasons, egg-laying by natural populations of *Melampus* takes place within about four days in each two-week cycle when their habitat in the upper salt-marsh is flooded by spring high tides (see Figs. 8 and 10). During the reproductive period of summer 1970, for example, there were four cycles of egg-laying at semilunar intervals in late May, June and early July. As we have confirmed in several summers (1965, 1966, 1967, 1968, 1970), stocks of *Melampus* brought into the laboratory maintain through the summer reproductive period the same pattern of semilunar egg-laying in the total absence of all tidal stimuli. There are other data (see Apley, 1970; Russell-Hunter *et al.,* 1970) which confirm that laying of egg-masses in phase with spring high tides is an obligate process. Physiological and ecological implications of this will be discussed in Section IV.

Under field conditions, this periodicity of egg-laying ensures that immediate desiccation of freshly laid egg-masses cannot occur. As first noted by Apley (1970), an even more important result of concurrence with the high spring tides is that the tidal flow through the "meadows" of *Spartina patens* and *Distichlis spicata* in the high salt-marsh (see Section IV, below) causes a redistribution of fine detritus and organic debris among the stems and bases of the plants. This detritus sifts over and covers the egg-masses with a layer which collects and maintains moisture around the eggs through the ensuing cycle of neap tides. In the field, undoubtedly it is the conditions ensured by this detritus layer which permit the survival and development of the eggs through the 11 or so days of nonsubmersion until the next spring high tides occur.

In the course of experimental work on hatching conditions (Section II, below), egg-masses were maintained on moist filterpaper in petri dishes stored at constant temperatures of either 18° C or 25° C. Development times did not vary greatly over large numbers of culture dishes. At 18° C, first cleavage takes place 5–7 hours after the egg-mass is laid, there is an early blastula by 12 hours, a true trochophore by the fourth day, and a well-differentiated and active veliger (Fig. 1B) by the eleventh day. Corresponding times at 25° C are trochophores by the third day, and active prehatching veligers by the ninth day.

II. Hatching, natural and experimental

In field populations of *Melampus,* the great bulk of egg-masses hatch about 13 days after laying when, in the natural course of the lunar cycle, they are once again subjected to several successive submergences. At these spring high tides, enormous numbers of newly hatched veligers (Fig. 2C) can be collected in the plankton of the seawater flooding the marsh. This massive and synchronous hatching of free-swimming veligers from the egg-masses is the second event in the

early life-history of *Melampus* to be confined (again for obvious adaptive reasons) to about four days in phase with spring tides. The rigid pattern found in synchronous egg-laying raises the question of whether the similarly synchronized hatching is obligate (dependent on an innate and rigorous developmental timetable) or facultative (dependent on the incidence of tidal submergence).

This question was the basis for an extensive series of laboratory experiments in summer 1970, and these demonstrated that there is greater potential flexibility in the time of hatching than could be deduced from the field data alone. Egg-masses laid in the laboratory were set out on moist filterpaper in petri dishes usually within six hours after laying. The filterpaper was moistened with a 50:50 mixture of distilled water and filtered seawater (corresponding roughly to the tonicity of the soil water in the high salt marsh during the days between spring
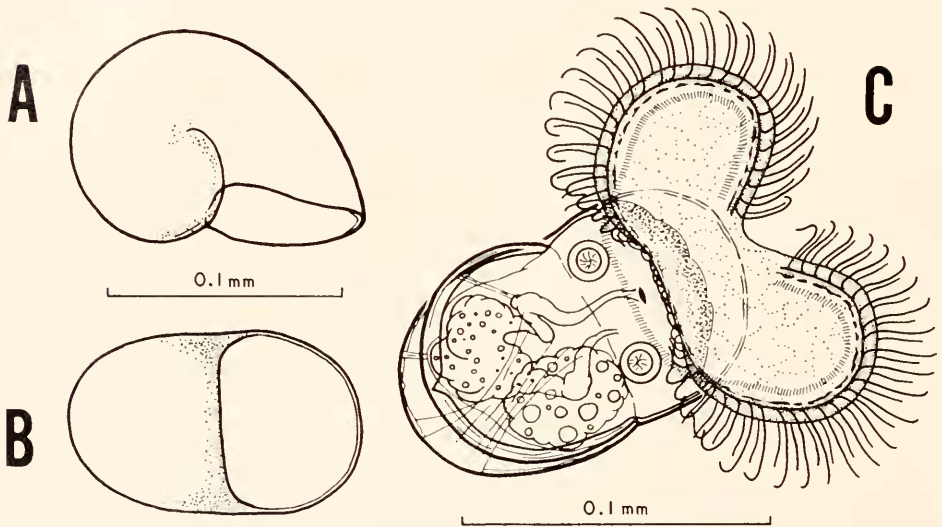

FIGURE 2. The veliger larva of *Melampus* at hatching: (A) and (B) two views of the simple planospiral veliger shell; and (C) a ventral view of a living veliger. For further description, see text.

tidal flooding). Care was taken to avoid having any excess water in the dishes (by daily checking) during their storage at constant temperatures of either 18° C or 25° C. Hatching of free-swimming veligers can normally take place only when masses containing appropriate developmental stages are flooded with seawater. The matrix jelly of egg-masses with eggs in *earlier* developmental stages has been found to behave almost like a perfect hygrometer and osmometer, swelling by uptake of free water especially if it is of reduced salinity, and shrinking proportionately upon desiccation. At later developmental stages (around 7.5–10 days at 18° C) flooding will cause premature eclosion (abortion) of larvae from a varying proportion of eggs in the mass. These larvae are not fully differentiated free-swimming veligers and their survival is doubtful. For still older masses, where complete flooding would produce successful veliger hatching, smaller amounts of ambient water in contact with the egg-masses (for example, a thin film of free

water over the filter paper in our petri dishes) will produce eclosion of veligers which then lie inactive on the jelly surface but can survive for a number of days (probably at least four) to become active and free-swimming upon appropriate flooding.

Apart from these preliminary timings and observations, our more quantitative experiments fall into three groups (Tables I–III). In all three series, numbered egg-masses were set up in petri dishes shortly after laying. Earlier laboratory observations had established that, although egg-masses can be deposited at all times of the day and night, about 90% are laid between midnight and 8 A.M. Examination by day of egg-masses laid in the field on the previous night reveals a majority of early cleavage stages with some blastulas, which suggests that the diurnal pattern of oviposition observed in the laboratory also occurs in natural field populations. The majority of the hatching experiments were set up around 10 A.M. when

TABLE I

Experimental hatching of Melampus *eggs: age at hatching of*
18° C and 25° C stocks with single flooding

Series	Date laid	No. of egg masses	Temp. (°C)	Date of flooding	Age at time of flooding (days)	Mean percentage hatching					Age at 90% hatching (days)
						2 hrs	8 hrs	24 hrs	28 hrs	48 hrs	
18/10M/A,F	7/10	20	18	7/17	7.3	0	0	0	20	55	9.3
25/10M/C,J,P	7/10	30	25	7/17	7.3	1	1	5	—	98	9.3
18/09M/A,F,M	7/09	30	18	7/17	8.3	0	0	0	10	73	10.3
25/09M/C,J,P	7/09	30	25	7/17	8.3	0	0	0	10	98	10.3
18/08M/A,F,M	7/08	30	18	7/17	9.3	0	0	0	10	98	11.3
25/08M/C,J,P	7/08	30	25	7/17	9.3	0	0	1	—	98	11.3
18/10M/B,G	7/10	20	18	7/20	10.3	0	0	0	94	—	11.5
25/10M/D,K,Q	7/10	30	25	7/20	10.3	4	13	30	93	—	11.5
18/09M/B,G,N	7/09	30	18	7/20	11.3	0	0	0	95	—	12.5
25/09M/D,K	7/09	20	25	7/20	11.3	1	5	6	94	—	12.5
18/08M/B,G,N	7/08	30	18	7/20	12.3	0	0	0	98	—	13.5
25/08M/D,K,Q	7/08	30	25	7/20	12.3	3	7	33	98	—	13.5

egg-masses laid since the previous midnight were screened and all showing later cleavage stages (or blastulas) discarded, along with any containing large percentages of abnormal, infertile or uncleaved eggs. Thus we began with each experimental group consisting of viable eggs at approximately the six-hour stage of development (or with a modal laying time of 4 A.M.). Normally 10, 20 or 30 egg-masses were set out for each time-temperature category in each experiment, and it should be noted that the percentages of hatching recorded in the tables were derived from a series of observations on *each* egg-mass which consisted of some 600 to 1100 eggs. Each record of percentage hatching for an egg-mass was based on about four detailed assessments of areas using a medium-power (× 45) dissecting microscope along with an inspection of the entire egg-mass. At times eclosion was seen to begin in one distinct zone of the egg-mass.

The first series of experiments, using egg-masses cultured both at 18° C and at 25° C establish the minimal ages at which viable veliger eclosion can occur as

a response to sustained flooding with seawater. The earliest successful hatchings in our material (see Table I) were from masses kept at 25° C where 98% hatching was achieved after 48 hours of flooding. These egg-masses were then at about 9.3 days development. At the more "normal" temperature of 18° C, > 90% hatching occurred after 48 hours of flooding in a few cases at developmental "age" of 10.3 days and more generally in cultures which reached 11.3 days at the end of the 48 hour period of flooding. Universally in cultures from both 18° C and 25° C > 90% hatching was achieved where the terminal "age" lay between 11.5 and 15 days.

Of course, such laboratory experiments where hatching resulted after a period of *continuous* flooding with seawater are somewhat unnatural. Two series of experiments (Tables II and III), more closely reflecting field conditions, involved

TABLE II

Experimental hatching of Melampus *eggs: effects of two advance "tidal" floodings at intervals of 12.5 hours (2AF) compared with control groups of the same ages*

Series	Date laid	No. of egg masses	Temp. (°C)	Treatment	Age at time of first flooding (days)	Mean per cent hatching after first flood	Mean per cent hatching after second flood	Mean per cent hatching following third and continuous flooding				Age at >90% hatching (days)
								2 hrs	8 hrs	24 hrs	28 hrs	
AF18/10M HO	7/10	20	18	2AF	12.3	0	0	20	30	68	93	14.5
18/10M/E	7/10	10	18	control	13.3	0	0	0	0	0	90	14.5
AF25/10M/LR	7/10	20	25	2AF	12.3	0	2	98	—	—	—	13.4
25/10M/I	7/10	10	25	control	13.3	0	0	5	30	50	90	14.5
AF18/09M/11	7/09	10	18	2AF	13.3	0	0	20	20	80	98	15.5
18/09M/EO	7/09	20	18	control	14.3	0	0	0	0	1	90	15.5
AF25/09M/L	7/09	10	25	2AF	13.3	0	25	98	—	—	—	14.4
25/09M IR	7/09	20	25	control	14.3	0	0	5	35	65	90	15.5
AF18/08M HO	7/08	20	18	2AF	14.3	0	0	35	45	98	—	16.3
18/08M/E	7/08	10	18	control	15.3	0	0	0	0	1	90	16.5
AF25/08M/LR	7/08	20	25	2AF	14.3	1	50	98	—	—	—	15.4
25/08M/I	7/08	10	25	control	15.3	0	0	30	30	85	90	16.5

"tidal bathing," in which the egg-masses were submerged for successive periods of one hour at intervals of approximately 12.5 hours (corresponding to successive spring high tides). The second series of experiments utilized two short (1 hour) floodings at tidal intervals (12.5 hours) followed by a third flooding continued to > 90% hatching (Table II). In the majority of cases (at a variety of ages from 13 to 16 days, and from development at 18° C and 25° C), > 90% hatching of veligers had occurred by 28 hours submergence in the "third flooding."

The third series of experiments was closest to field conditions, and in it the egg-masses were again submerged for four successive periods of one hour at intervals of 12.5 hours. Timings were arranged so that the fourth floodings corresponded to the twelfth to fourteenth days of development, and only healthy egg-masses kept at 18° C were used (Table III). Under these conditions, the first and second floodings had no overt effect and the third flooding produced

< 5% or *no* veliger hatching regardless of "age." In cases where an age of 14 days had been reached, the fourth flooding was usually completely effective and always produced appreciable hatching in 30 minutes and > 98% hatching if allowed to continue for 1.5 hours. There was a tendency where ages of 12 and 13 days had been reached for hatching to be less complete (< 50%–85%) and to take somewhat longer (> 2 hours) in the fourth flooding. It should be noted that the total time immersed in water in these experiments (for nearly complete hatching at age of 14 days) is only 4.5 hours. This is less than one-tenth of the required time submerged (48 hours) in the experiments involving sustained flooding, and much more closely comparable to natural tidal conditions in the field. However it should also be noted that the elapsed time in *all* the successful experiments was about 48 hours.

TABLE III

Experimental hatching of Melampus *eggs: effects of four successive "tidal" floodings each of one hour's duration*

Series	Date laid	No. of egg masses	Temp. (°C)	Age at first flooding (days)	Mean percentage hatching			Mean percentage hatching, fourth and continuous flooding, elapsed hours			Age at 90% hatching (days)
					first flooding	second flooding	third flooding	1 hr	2–3 hrs	9–12 hrs	
18/10M/S	7/10	10	18	10.5	0	0	1	17	90	90	12.0
18/10M/T	7/10	10	18	10.5	0	0	0	8	60	60	—
18/10M/U	7/10	10	18	10.5	0	0	0	18	75	75	—
18/10M/V	7/10	10	18	11.0	0	0	1	2	5	98	13.0
18/10M/W	7/10	10	18	11.0	0	0	1	2	5	98	13.0
18/10M/X	7/10	10	18	11.0	0	0	1	2	5	98	13.0
18/08M/S	7/08	10	18	12.5	0	0	4	20	98	—	14.0
18/08M/T	7/08	10	18	12.5	0	0	4	20	98	—	14.0
18/08M/U	7/08	10	18	12.5	0	0	4	35	98	—	14.0

The veligers in egg-masses raised in the laboratory proved to retain some viability over an extraordinarily long time span. Over-extension of a few experiments showed that viable veligers could still hatch from egg-masses maintained at 18° C 23 days after laying. Our most extreme record of egg-masses (maintained at 18° C but not carefully kept and probably somewhat dried out on occasion) yielded about 80% hatching of veligers after 44 hours of flooding and at 42 days after laying. This extraordinarily long viability could have some adaptational significance in natural populations. The highest level reached by successive sets of spring tides varies throughout the year and, in the habitat of *Melampus* (see Section IV), it would be possible for eggs to be laid after spring tide flooding at a level which would not be submerged in seawater again for 56 days (or four complete cycles of spring and neap tides). Obviously few adult snails are ever found at such levels of the salt marsh and fewer still will lay eggs there, but the flexibility of hatching age and the extreme viability of eggs after they have reached the veliger stage of development could accommodate such occurrences.

From all this, it is clear, that unlike the rigid semilunar pattern of egg-laying, the synchronous tidal occurrence of hatching in *Melampus* is not obligate. The developmental age at hatching is flexible—but eclosion will usually be brought about from egg-masses of 10 to 24 days by a *sequence of about four tidal floodings in under 50 hours*. Being facultative, the process allows better survival and overlap of cohorts but it also re-establishes the synchronization. As will be discussed later, this is highly significant for the future synchronous resettlement of veligers into the appropriate levels of the salt-marsh habitat at a later cycle of spring tides.

During the experimental work on hatching, details of the process of eclosion were followed on a number of occasions. Since the detailed papers of Davis (1964, 1967, 1968) on hatching processes in various invertebrate eggs do not cover any ellobiid snails, it is worth briefly reporting here on the mechanism of hatching in *Melampus*. The first visible sign of hatching is a wrinkling and loss of rigidity of the egg-shell where it overlies the active cilia of the velum of the contained larva. This wrinkling can begin at either the blunter or the more pointed end of the egg (see Fig. 1B) but is always initiated over the velum. The wrinkling seems to be caused by some process internal to the egg-shell, and could result from the detection by the contained veliger of some earlier stimulus to hatching. It is tempting to suppose that certain elapsed changes in the osmolarity of the jelly matrix of the egg-mass provide this stimulus. As the crumpling spreads and extends over the entire surface of the egg-shell, the velar end of the egg is ruptured by vigorous thrusts of the velum and head region. It is worth noting that, after the wrinkling has begun, all the subsequent stages of hatching can be explained as mechanical. The veliger is not able to move its cilia effectively until it has freed the velum of remnants of softened egg-shell fragments. Final separation of egg-shell and veliger is aided by the allochalazal strand, an anchoring device, which allows the now effectively swimming veliger to pull away from the clinging mass of egg-shell. Before hatching the allochalazal strand can be seen to run from one egg-shell to another between which it is embedded in the concentric membranes of the spherical envelope (Fig. 1A). The attached strand which survives the softening and crumpling process of early eclosion is, of course, at the opposite end of the egg-shell. Once free of the egg-shell, other envelopes and the jelly matrix offer little resistance to the veliger which, by vigorous movements of the operculum and beating of the velar cilia, can swim free of the egg-mass in less time than it took to escape the egg-shell. Hatched veligers then become relatively inactive unless there is a sufficient volume of seawater available for active swimming and feeding. Under natural circumstances, they swim actively and are swept out to sea from the salt marsh with the ebbing spring tide to become part of the inshore plankton.

III. Mantle-shell changes in early growth

On hatching, the relatively small "primitive" egg of *Melampus* yields a small veliger (Fig. 2C). Apart from the ecological difficulties and the synchronization of the shifts into and out of the salt-marsh habitat (Sections II and IV), there have to be major changes in the patterns of mantle-shell growth to accommodate the anatomical and behavioral transitions from ciliated planktonic larva to spat and young adult. The latter shift involves a true metamorphosis of mantle and shell,

This can be stated quite simply: the larval and postlarval shells in *Melampus* are sinistral in coiling and low-spired (nearly planospiral), while the spat and adult shells are dextral and truly turbinate. Snail shells showing evidence of such reversal of the spiral axis of growth have long been termed heterostrophic. There are a number of early records of heterostrophy in the Ellobiidae (Fischer and Crosse, 1900; Pelseneer, 1901, 1906; Harry, 1951; see also Morton, 1955a, b, and references therein), but only one illustrated description (Harry, 1951) of a single juvenile shell in a species of *Pythia* from the Philippines. We can now provide, for *Melampus,* the first detailed account of the stages of development of an ellobiid heterostrophic shell. The functional and ecological implications, both of the mantle-shell metamorphosis and of the other, more gradual, changes in growth gradients in early stages of *Melampus,* are not without evolutionary significance.

The newly hatched veliger of *Melampus* (Fig. 2C) has a shell of about 125 μ maximum diameter (Figs. 2A, B) which appears almost planospiral (that is, indistinguishable as regards sinistrality or dextrality because of the lack of an apex). As in the majority of snails which hatch as veligers, the mantle rudiment and the
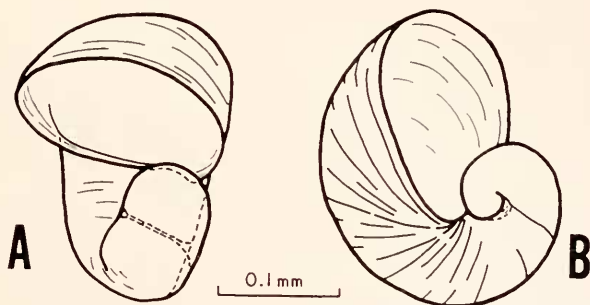

FIGURE 3. The shell of a veliger of *Melampus* after fourteen days of planktonic life. The shell coiling now appears moderately turbinate and sinistral, and the original prehatching shell can be distinguished in 3B. For further discussion, see text.

shell which it secretes are well-differentiated long before hatching. When eggs developing at 18° C are followed, we have a true trochophore by the fourth day and, within the next 24 hours, the first mantle rudiment becomes distinguishable on the visceral mass while the other half of the now "waisted" embryo differentiates into head-foot-velum rudiments. By the sixth day, when torsion has occurred, the mantle-shell rudiment is clearly defined and cup-shaped, and a tiny but obvious opercular rudiment appears on the posterior part of the foot. From the seventh to the tenth days, most active growth of the mantle occurs around that arc of its edge facing the velum, generating (by this simplest possible of pallial edge growth gradients) the planospiral shell (Figs. 1B and 2ABC) of the veliger at hatching. This veliger shell is almost entirely proteinaceous and little secretion of calcium carbonate has occurred. Veligers of mean tissue dry weight 129 ng had shell calcium carbonate values of less than 13 ng, which can be compared with early settled spat stages of *Melampus* where shell calcium makes up half of the dry weight (Russell Hunter and Apley, 1966).

As the veliger grows in the plankton, the shell coiling becomes turbinate (that

is, no longer has a spiral axis in one plane) and appears sinistral (Fig. 3AB).
[Throughout this section, for convenience of description, the terms sinistral and
dextral are used only in their long-established *descriptive* sense as applied to the
external appearance of turbinate coiled shells. Thus sinistral refers to coiling
which appears to grow anticlockwise when viewed from the point of origin,
dextral to apparently clockwise growth. Their *morphological* sense, as regards
the orientation of the heart and other asymmetric organs in gastropod anatomy, is
not implied here. The metamorphosis which we are about to describe is one of
shell-coiling reflecting a shift of the growth of the mantle from sinistrality to dex-
trality; it implies no changes in living structures other than the actively growing
pallial edge; and it certainly does *not* involve shift to a condition of *situs inversus*
for the heart, the kidney and all other unpaired structures. Our use of sinistral
and dextral in these descriptions will be related to the morphologically more cor-
rect terms hyperstrophic and orthostrophic only in the final discussion.] The
veliger shell illustrated in Figure 3AB is about 245 μ in maximum dimension, shows
sinistral coiling, and has a rapidly enlarging aperture to accommodate the large lobes
of the velum at this stage. Since the shell has "grown" by marginal increments laid
down by the secretory cells of the mantle-edge, it is clear that we have had some
allometry here in the growth gradient pattern of cell-divisions at the pallial edge.
The shell of Figure 3AB is from a veliger of 14 ± 1 days after hatching, grown in a
laboratory culture. Similar late veligers were found occasionally in the inshore
plankton, but neither culture nor field material was available in sufficient quantity
for any analyses of organic carbon or of shell calcium. However, manipulating
these tiny shells suggested that some calcification had occurred since hatching.
It is worth noting that veligers of this age retain the operculum which has become
greatly enlarged since hatching. The majority of prosobranch snails have an
operculum througout life, and even those with limpet-shaped shells and no oper-
culum as adults, such as *Acmaea* and *Crepidula*, have a larval operculum as veligers.
In the two subclasses of "higher gastropods," *all* adult pulmonate snails (and
almost all opisthobranchs) lack the operculum. Only in a few ellobiid genera like
Melampus is there a temporary operculum in the veliger stage. Although this may
have some phyletic significance, it is worth putting forward a simple functional
explanation for the retention of the operculum at this stage, again based on the
need to accommodate the large velar lobes. For a non-operculate snail such as a
pulmonate or shell-bearing opisthobranch to be fully protected by the shell, the head-
foot must be withdrawn well beyond the edge of the shell at its aperture, and thus
the internal volume or capacity of the shell must be considerably in excess of the
total volume of the tissues. This need not be so in a snail with an operculum
which can close to an accurate fit with the edge of the shell at its aperture. The
fact that the adaptational significance of withdrawal to the veliger may involve the
rapid "switching-off" of its locomotory cilia as well as "protection" (Garstang,
1928) merely emphasizes the advantages conferred by the operculate condition
in terms of the economy of shell-growth. We have some records of veligers re-
tained alive in culture (without settlement) for 20 days, by which time the shell
had grown to a maximum of 276 μ though the latter part of the shell-growth
involved the slower enlargement of the aperture typical of the postlarva and was
unlike the earlier veliger "flare." We assume, however, that Figure 3AB is
more typical of the form of a late veliger shell just before settlement.
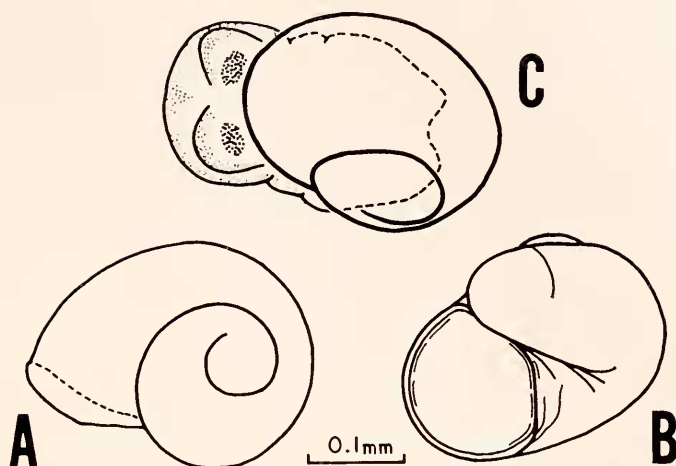
FIGURE 4. The "postlarva" of *Melampus* after settlement but before metamorphosis: (A) and (B) two views of the sinistral turbinate shell; and (C) a dorsal view of a living specimen collected in the salt marsh after settlement.

After this stage, settlement occurs (back into the salt marsh, see Section IV) and the postlarva quickly loses both velar lobes and operculum, as it takes up a crawling rather than a swimming mode. The maximum shell dimension at settlement lies between 270 μ and 290 μ. Shell metamorphosis does *not* occur at this time. The shell as it appears a few days after settlement is shown in Figures 4A and 4B. It is about 290 μ in maximum dimension, still shows sinistral coiling, but recent increments to the edge have involved *isometric* growth gradients in the mantle-margin (that is, during the last half-whorl of the enlargement of the shell and of the slower enlargement of its aperture, the *shape of the aperture has not changed*). The shell is now more obviously calcified, but again we have no quantitative analyses for this stage. A living specimen of this premetamorphic postlarval stage, collected in the field at Sippewisset, is illustrated in Figure 4C.

Soon after this, the metamorphosis in the growth of the mantle tissues occurs, the immediate result of which is the initial secretion of a spat shell which shows
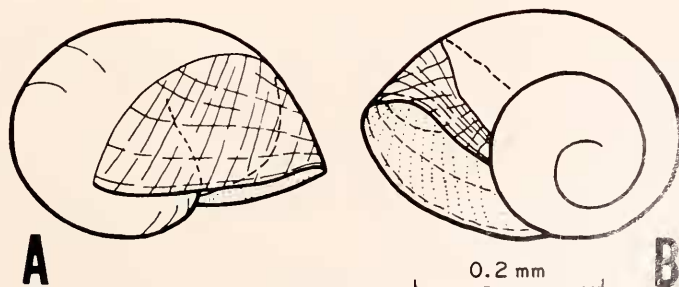

FIGURE 5. The first shell growth after metamorphosis of the mantle in *Melampus*. For further discussion, see text.

the new dextral coiling which will be retained throughout spat, and juvenile and adult growth (Figs. 5AB, 6ABC). The first shell secretion after metamorphosis is shown in Figures 5A and 5B. The reorientation of the spiral normal axis of growth can better be seen by comparing the slightly older postmetamorphic spat of Figures 6A and 6B, with the orientation of the earlier postlarval shell (or protoconch) at the apex of the later juvenile shell shown in Figure 6C. All these later illustrations (Figs. 4–6) are based on field specimens collected at appropriate times in Sippewisset marsh, and are previously undescribed. Appropriate comparisons of these figures show the shift from a sinistral to a dextral shell-coiling. As has often been pointed out, the growth and subsequent configuration of the gastropod shell can be conceptualized as consisting of
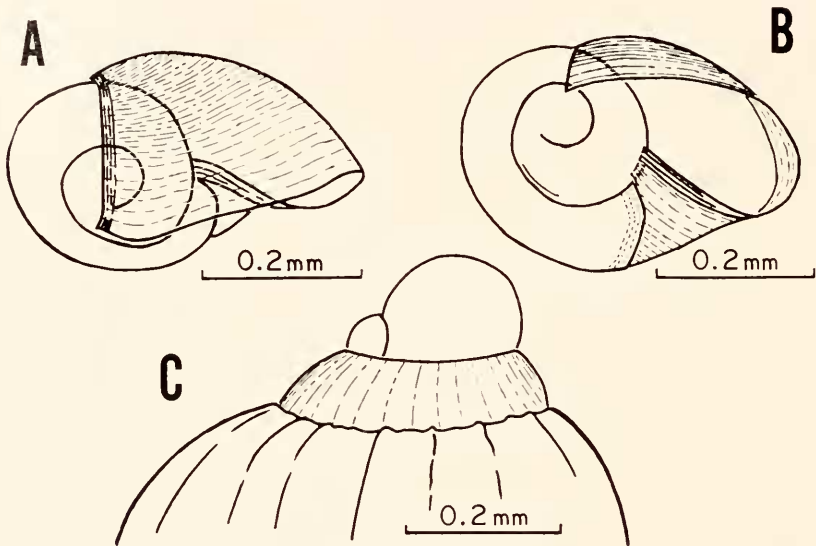

FIGURE 6. Metamorphosis of mantle-shell in *Melampus:* (A) and (B) two views of the shell of a postmetamorphic spat (slightly older than that illustrated in Fig. 5); and (C) the postlarval shell or protoconch at the apex of a later juvenile shell. The sinistral growth of the postlarval shell is replaced by a dextral shell coiling which persists in juvenile and adult. For further discussion, see text.

two somewhat distinct growth processes (Huxley, 1932; Russell-Hunter, 1953a, 1968; Fretter and Graham, 1962; Wilbur and Owen, 1964). One is growth along a spiral normal axis which can be visualized as running from the origin of the spiral (the apex of the shell in most cases) to the centroid of the plane of the aperture of the shell (sometimes more loosely defined as the midpoint of the foot). The other is the continued expansion of the mantle (and therefore of the shell which it secretes) by marginal increments to accommodate growth of the body. As a result of this accretionary method of growth, the form of the shell may be described in terms of the growth gradients of the mantle edge which usually are consistently maintained through each period of growth. Biologically, of course, the twofold conceptualization is somewhat false: the properties of the

spiral normal axis (including the apical angle of its visualized cone) are not entirely independent of the growth gradients of the pallial aperture. Both are generated by the differential rates of cell division in the cells of the mantle edge.

Metamorphosis in *Melampus* involves an abrupt change by about 90° in the *direction* of the spiral axis and the initiation of an *entirely new pattern* of growth gradients for the mantle-edge (the new pattern being a slightly distorted mirror-image of the older one). All the other changes of shell shape in *Melampus* reported and illustrated here can be described in terms of sustained gradients between the rates of growth at points around the mantle-edge (allowing the aperture shape and the proportions between major dimensions to remain unchanged with growth, or isometry) or in terms of proportionally changing gradients between these rates (or allometry, as in the shell growth of the veliger after hatching). Such description of the mantle-shell metamorphosis in *Melampus* is not possible, and the
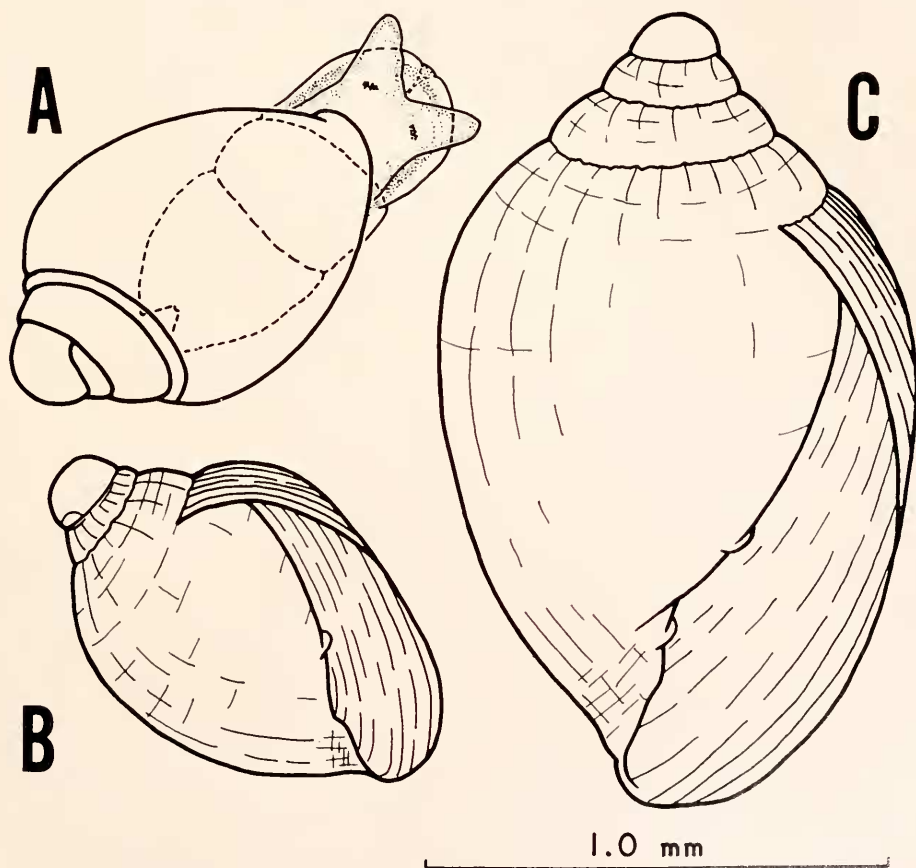

FIGURE 7. Later spat stages of *Melampus*: (A) dorsal view of a living specimen of a young spat about 4 to 5 weeks after settlement; (B) the dextral turbinate shell of a similar spat; and (C) shell of a spat about 8 weeks after settlement, in which the adult aperture shape (reflecting the growth gradients of the mantle-edge) is already established. Later shell growth is isometric, see text.

metamorphic process involves an abrupt change in the form of the annulus of dividing pallial cells which generates both differential shell increments and the growth spiral.

Soon after metamorphosis, a near-adult pattern of growth gradients is established in the mantle-edge. This can be seen by comparing the shell aperture in Figure 7C, of a 1.6 mm spat where the adult shell shape is already established, with the 400 μ postmetamorphic young spat of Figure 6B. Despite the fourfold increase in linear dimensions the shape generated by the pallial edge does not change: later shell growth is isometric.

A living young spat of about 1 mm shell-length collected in the field about four to five weeks after settlement is shown in Figure 7A. Batches of closely comparable spat, though somewhat smaller at a mean shell-length of 675 μ, provided some biomass data. Mean tissue dry weight is about 11.3 μg (mean for 8 batches) and mean organic carbon 5.03 μg (mean of 6 determinations), values about a hundred times those already reported for newly hatched veligers. As pointed out in an earlier note (Russell Hunter and Apley, 1966), in *Melampus*, growth (in any real biomass terms) extends through three orders of magnitude in the first three months of life and through nearly six in the entire 3–4 year lifespan. In the spat of mean length 675 μ the shell is now well-calcified with mean calcium carbonate at 11.9 μg (mean of 8 determinations) or over 50% of the dry weight (shell included). Similar cases of a shift from a non-calcareous and largely proteinaceous shell in the veliger to a largely calcareous shell in settled spat for marine prosobranch snails are discussed by Fretter and Graham (1962). Further aspects of the growth of spat and of their population dynamics will be set out in Section IV.

IV. Ecology of early life-history

Zonal distribution. Natural populations of *Melampus* are found in the higher levels of salt marshes. The zone, within which living animals are numerous and where all reproduction occurs, can readily be defined in terms of vegetation and soil, or in terms of the vertical excursion of the tides.

The natural vegetation zones in the upper levels of the salt marsh at Little Sippewisset are characteristic of those found throughout New England (New Jersey to Maine). Some marshes, where populations of *Melampus* have been studied by us, are much more extensive (for example at Lawrence, Long Island and at West Barnstable, north Cape Cod) but, although the zones of plants are of much greater horizontal extent in such marshes, the vertical distribution of plant species conforms to much the same pattern (see Chapman, 1940; Blum, 1968). At the landward edge of the marsh are stands of *Phragmites communis* and *Typha angustifolia* (cat-tails) with variable amounts of woody xerophytic bushes, such as *Iva frutescens* var. *oraria* (marsh-elder), *Myrica pensylvanica* (bayberry) and *Prunus maritima* (beach-plum). *Melampus* rarely moves into, and must never linger in, this zone. Woody glasswort (*Salicornia virginica*) occurs just below this level, fiddler-crabs (*Uca pugnax* and *U. pugilator*) are often abundant, and this is the uppermost level for populations of *Melampus*. There is next a patchy intermediate zone of the high-water species of "soft" marsh-grass, *Spartina patens*, of the rush *Juncus gerardi*, and of the spike-grass *Distichlis*

spicata, the former two species covering the better drained patches. Then there is a slightly lower, slightly wetter, zone where *Distichlis* is dominant, with some *S. patens* and with occasional clumps of the rush, *Juncus gerardi,* and of another glasswort, *Salicornia europaca.* In this zone, the soil may still dry out sufficiently for *Uca* spp. to burrow. In this *Distichlis* zone, as in the *S. patens-Juncus-Distichlis* zone above it, *Melampus* is abundant, and it is largely in these zones that copulation and egg-laying occurs at appropriate spring high tides. As discussed in detail by Blum (1968), it is in these zones that the algal community of the soil surface is characterized by the dominance of a single species of *Calothrix.* Below these zones, and in clearly wetter conditions where the soil water is near
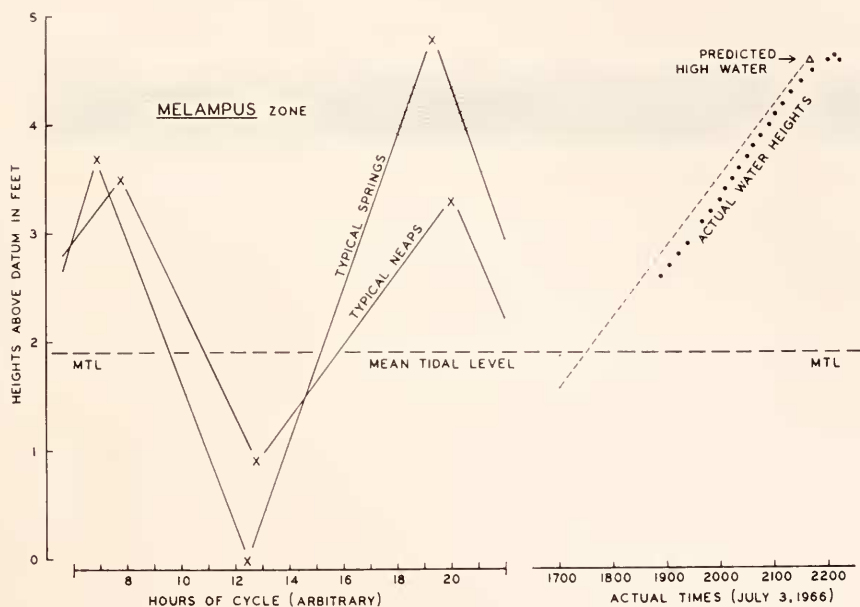

FIGURE 8. The relation of the vertical zone (3.9 to 4.4 feet above datum) occupied by natural populations of *Melampus* at Little Sippewisset, typical ranges of spring (−0.02 to 4.77 feet) and neap (0.9 to 3.28 feet) tides, and the observed time course of actual tidal heights during one high water of springs. Note that the mean level of all tides (MTL) does not necessarily correspond to mean sea level (MSL).

the surface even at low tides and there are often superficial puddles, is a zone of the "dwarf" growth form of the major marsh-grass species, *Spartina alterniflora.* *Melampus* occurs in this zone, but does not normally lay eggs in the wetter, lower parts of it. As noted above, it is in this zone that spat of *Melampus* may seem to be most numerous during the first four to six weeks after settlement. Lower still are the taller stands of *Spartina alterniflora,* mostly three to four feet in height, principally along the edges of the small drainage channels and larger tidal creeks which dissect the marsh. *Melampus* does not occur in the drainage channels (or at any *lower* tidal level), and is only rarely found among the roots of the tall form of *S. alterniflora.* As this present manuscript was being revised, Redfield (1972)

provided a detailed account of the ontogeny and plant ecology of the salt marsh at West Barnstable.

In summary, in terms of plant zonation, the populations of *Melampus* are found principally in the *Spartina patens-Juncus-Distichlis* zone, in the *Distichlis* zone, and in the upper levels of the zone dominated by the "dwarf" growth form of *S. alterniflora*. Under slight differences of drainage, the populations of *Melampus* can extend a few centimeters (actually about 0.25 feet) vertically above and below these zones. (In many marshes, of course, a few centimeters vertical extension could imply the horizontal colonization of hundreds of meters of marsh.)

Figure 8 shows the relationship of the zone occupied by *Melampus* at Little Sippewisset to the time course of the upper part of a spring tide cycle. In general terms, the range of normal spring tides will encompass all but a small number of extreme tides (perhaps all but six cycles in any year). If that range of springs is arbitrarily divided into eight zones of equal vertical extent—with four of them lying above the mean level of all tides (which does not necessarily correspond to mean sea level), then extensive populations of *Melampus* are almost entirely limited to the uppermost of these vertical zones (that is to the upper quarter of the shore lying above mean tide level). *Melampus* may extend downward through the next highest level, and upward to the extreme upper level bathed by *any* tides, but it is only abundant in, and only reproduces in, that uppermost eighth of the normal spring tidal range (or, at Little Sippewisset, 3.9 to 4.4 feet above datum). The figure shows the predicted curves for typical high tides of neaps and of springs at Sippewisset, and the observed water levels corresponding to the latter. On the evening of the observations, offshore winds caused a delay of about twenty minutes in the rise, but no apparent distortion.

Another way of putting it is that the populations of *Melampus* are largely found in the upper two thirds of the zone lying above the mean high water of neap tides (MHWN) and below the mean high water of spring tides (MHWS), but are also found in the zone lying between MHWS and the extreme upper limit washed by any tides. Thus they live in the upper half of what has been termed for rocky shores the *supralittoral fringe*, and defined as "the region within the littoral zone not wetted by all tides" (Stephenson and Stephenson, 1948; Southward, 1958, Table I, page 141).

Egg, larval and postlarval ecology. Our populations of *Melampus* live in the upper 12% of the intertidal zone, and some may be bathed by seawater for only 8 hours out of the 354.4 hours (or 2.3%) of each semilunar tidal cycle. Adults of *Melampus* do *not* migrate down to lower zones of the littoral for reproduction, as do certain arthropods of the supralittoral fringe. *Melampus* fits its peculiar environment by achieving strict synchronization with spring high tides for its processes of (a) copulation and egg-laying, (b) hatching, and (c) veliger settlement, and possibly some looser synchronization of intermediate stages of the life-history such as postlarval metamorphosis and early spat growth. These adaptational achievements of temporal "fitting" are the dominant aspect of behavior and ecology in *Melampus*.

As first elucidated by Apley (1967, and unpublished), the onset of the overall period of reproduction for these populations of *Melampus* in late-spring–early-summer is determined by changing day-length as one *essential* signal in a complex

environmental input. This input includes conditioning temperatures and individual snail biomass with nutritional state, as well as day-length. In the laboratory, Apley (1967) was able to induce copulation and egg-laying in cultures during the month of January, by holding them at 22° C and applying day-length conditions of L16D8 and L14D10. Application of L12D12 under otherwise identical conditions did not induce reproduction. In these experiments there was a lag, or latent period, of about 20 days.

For the latitude of our populations at Sippewisset, the day-lengths of 12, 13 and 14 hours (L12D12, L13D11, and L14D10) are reached in spring on March 17, April 7 and April 30, respectively. Were it not for the importance of field temperatures, we might postulate that the field onset of reproduction might be set by the date of April 7 with a latent period of about 27 days (the extra 7 days to approximate the fit to the semilunar cycle). Thus theoretically, if field temperatures were around 18–20° C, we might expect the earliest reproduction around May 4. In fact, the field temperatures for the months of April and May at Sippewisset range from 3 to 13° C and from 6.5 to 19° C, respectively, and our earliest field observations of the aggregations, which precede copulation and egg-laying, have been on May 26 (1968) and on May 24 (1970).

In different years, the field onset of reproduction at Sippewisset has come at an appropriate day of spring tides in late May or early June. There are normally three cycles of egg-laying (occasionally four, see Table IV) in each annual reproductive period (Apley, 1967, 1970; Apley *et al.*, 1967). Thus this annual period always extends from late May or early June through early July.

Each breeding cycle within the reproductive period shows a definite semilunar periodicity with egg-laying confined to four days in phase with the spring tides. Taking full or new moon as day 0, the patterned behavioral sequence involves aggregation (day −1), copulation (day +1), egg-laying (days +2 through +6), and dispersion (days +6 through +8). This is shown as part of Figure 10. As already noted, stocks of *Melampus* brought into the laboratory from April onwards will maintain the same semilunar pattern of reproductive behaviour as the undisturbed field populations at Sippewisset.

In the reproductive period of 1970 in the salt marsh at Little Sippewisset, there were four cycles of egg-laying around the modal dates of May 24, June 7, June 22, and July 6. Each of these dates is about three days after a new or a full moon (that is, day +3 in the behavioral schedule set out above). The third cycle of egg-laying (around June 22) was considerably less productive than the massive egg-layings of June 7 and July 6.

Peak natural hatching of veligers occurred in the field with the spring tides of about 13 days later, actually on June 6, June 20, July 5, and July 19. Our regular townet collections were taken in the creek which drains the salt marsh during the two hours immediately after high tide, and thus they sampled the veligers being swept out to sea from the marsh. Such veligers were invariably newly hatched; older veligers were never collected at such times. The lunar phases in 1970 corresponding to these hatchings were new moon on June 4, full, June 19; new, July 3; and full, July 18. Some egg-masses must have remained unhatched in our field areas, because later plankton collections allowed us to deduce that some hatching occurred around August 2 (new moon), and we col-

lected some apparently newly hatched veligers in the field on August 16 (full moon, August 17). This shows that the extraordinary viability of egg-masses in the laboratory hatching experiments (Section II), when kept out of free water for 23 and 42 days, is not artificial but parallels field conditions. It is unfortunate for our hypothesis that such egg-masses showing delayed hatching must have been laid in the highest levels of the marsh. While some of the highest spring tides of the entire summer came with the full moon at August 17, the spring tides of the new moon at August 2 were less extensive than any from May 1 through September 1, 1970.

Since the hatched veligers drift out into the general inshore plankton, we have been unable to establish the earliest larval growth rates from field samples. In laboratory cultures, the veliger of *Melampus* first feeds within one hour of hatching. As in the better known prosobranch veligers (Fretter and Graham, 1962; Fretter, 1967; Mapstone, 1970; Pilkington and Fretter, 1970) the same action of the preoral velar cilia propels the larva forwards, collects particulate food, and concentrates the particles in the food-groove. Both the preoral band of longer cilia (derived from the prototroch of the earlier trochophore larva) and the postoral band of shorter cilia (metatroch of the trochophore), which lie on either side of the food-groove, are essential to the feeding mechanism, though perhaps not to veliger locomotion. The food-groove, bounded by its continuous "fences" of the prototroch and metatroch, runs round the margin of both velar lobes (Fig. 2C) and the mouth lies within it on the ventral side. As recently clearly elucidated for the feeding mechanism of trochophores (Strathmann, Jahn and Fonseca, 1972), the opposed beat of the two ciliary bands is responsible for most effective collection of particles. The veliger of *Melampus* is small with a moderately proportioned velum (Fig. 2C) not as large as the velar "wings" of nassariid or naticid veligers, but proportionately larger than those of some littorinids. Functionally it is a relatively continuous swimmer (and therefore food intake is potentially continuous), unlike the veligers of littorinids whose cilia frequently stop for a few seconds. Fed on cultures of *Dunaliella tertidecta* in the laboratory, in conditions far from optimal, we had veligers hatching at 125 μ (shell maximum dimension) regularly reaching 185 μ in eight days (corresponding to rates of 7.5 μ per day, or an increase in mass of about 3.38 times). The field rates must be somewhat higher since the newly hatched veliger at 125 μ reaches its presettlement size of 270 μ in fourteen days (corresponding to 10.4 μ per day).

The largest veliger of *Melampus* that we have found in plankton samples measured 288 μ and our largest cultured veliger was 276 μ (although it was then 20 days from hatching). As already noted, settlement occurs in larvae of between 270 μ and 290 μ. However, our largest cultured veligers had already moved to the postlarval pattern of shell growth (that is, had shells more like Fig. 4A than Fig. 3B). Some observations on such "late" veligers are worth reporting. In life this stage is, in many features of structure and function, intermediate between the free-swimming veliger (Fig. 2C) and the early post-larva (Fig. 4C). It swims reasonably well for short periods, although it looks awkward and as though the relatively small velar lobes are inadequate to lift the new relatively massive visceral mass and shell. It is also capable of crawling, and can move on its well-developed locomotory foot as competently as a spat of 1.2 mm shell-length.

However, the head-foot organization is markedly different from that of the post-larva or spat: the foot is much narrower and more elongate anteriorly (the eyes lie very far back) and posteriorly (where there is a long tail supporting the retained operculum). This intermediate anatomy and behavior may be typical of veligers at settlement although the majority of them will have a shell form closer to that of Figure 3B.

Although our evidence can never be more than circumstantial, we are reasonably sure that the veligers spend about fourteen days in the plankton. In 1970 considerable settlements must have occurred with the spring tides of July 4 and August 2 (corresponding to egg-laying of June 7 and July 6). Sets of spat samples taken in mid-August (see next section) established that, although a fraction of the
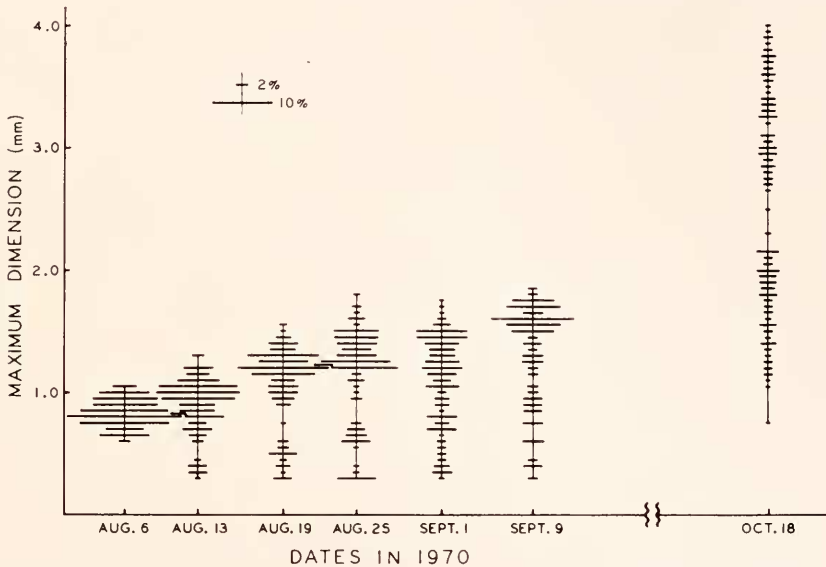

FIGURE 9. The growth of spat stages of *Melampus*. Sets of histograms show size distributions in successive samples of spat from Little Sippewisset in late summer, 1970. Sampling is probably slightly biased against spat of under 0.6 mm maximum shell dimension. Sample numbers ranged from 47 (Aug. 6) to 149 (Aug. 19), but the histograms indicate the percentage of the sample in each class interval to facilitate comparison.

total settlement can take place below the *Melampus* zone, the bulk of the return takes place into the exact zone occupied by the adults and that the differences in distribution which occur can be explained on mechanical grounds of water flow. For example, denser settlement is found among *Spartina* roots along the edges of drainage channels, where some filtering effect (along with increased contact stimuli to the veligers) must occur as each spring high tide recedes from the marshes. Other typical sites for denser settlement are the centers of shallow depressions within the *Melampus* zone, where again concentration of veligers could occur on a falling tide.

We know that growth of the postlarva immediately after settlement is slow and limited (see Fig. 4 and Section III), and that the remnants of velar lobes

and the operculum are lost very quickly. The postlarva shows cryptic responses in marked avoidance of light and air currents at this time. Circumstantial evidence (from the dates and growth of early spat collections) suggests that there is a further slowing of growth rates in the postlarva for the 10 to 14 days preceding metamorphosis. The first true spat (postmetamorphic as in Fig. 5A and B) are around 320 μ in maximum dimension with only a small gain from the early post-larva (Fig. 4C at 290 μ maximum dimension). Timings for settlement and for the postmetamorphic appearance of true spat are also shown diagrammatically in Figure 10 for a three-cycle period.

Spat ecology. It is obvious that direct collection of early spat from the field is almost impossible although, on a number of occasions, "long-arm" mounted dissecting microscopes and suitable illuminators were taken into the field for direct observations of the soil surface in the high salt marsh. In our regular sampling for spat, we cut discs of 9.6 cm diameter from the appropriate levels of the habitat, taking about 3.5 cm depth of soil but shearing off the grasses at less than 1 cm above the soil surface. These discs could be taken to the laboratory, the soil surface between the stubble examined under a low-power dissecting microscope, and all spat picked off using a fine camel-hair brush. Initially, various methods of applying heat and of watering were used to bring spat to the surface, but our standard method involved repeated scanning at laboratory temperatures, relying on a high intensity spotlight to detect spat moving in the tangled algal mat at the soil surface. After completion of sampling, the turf discs were returned to their original positions in the field.

Successive size distributions for spat samples are shown in Figure 9. The youngest postmetamorphic spat are about 320 μ in maximum dimension and, although there is some recruitment to our samples at this level (August 13 through September 9), our techniques may be slightly biased against spat of under 600 μ. However, it is almost certain that the size distribution of spat at August 6, 1970 (Fig. 9) is made up almost entirely of the first of the two major settlements of the summer, that of July 4. Over the 33 days since settlement, these spat have grown from the modal size of 280 μ at settlement to the postmetamorphic size of about 320 μ in some 14–15 days and then to a modal spat size of 800 μ in the following 18–19 days. As can be seen from the figure, the rate of spat growth remains high: a further five weeks of growth taking the median members of this particular cohort to a size of 1.6 mm. The other major settlement of the summer, that of August 2, is represented in the size distributions from mid-August onwards but no clear picture either of two or of four distinct cohorts emerges in the samples of September or October. The cohorts are blurred partly by the other smaller but synchronized settlements of June 21 and July 18, and by any later settlements corresponding to the delayed hatchings of August 2 and August 16 (that is settlements which might yield spat sample recruitment around August 30 and September 12). There is some evidence that the spat derived from later settlements grow more slowly, which could decrease survivorship in later cohorts (see Discussion below). As already noted (Section III), the first four weeks of growth after settlement can yield spat of mean tissue dry weight around 11.3 μg (at a shell length of 675 μ), a hundred times the tissue weight of the newly hatched veliger. For the earliest cohort discussed above (Fig. 9) the

median size of 1.6 mm reached by September 9 (that is, about nine weeks after settlement) would correspond to a mean tissue dry weight around 185 μg, a further 16-fold increase in real biomass terms. Thus, for that cohort of veligers hatched at the optimal time, biomass growth can extend through more than three orders of magnitude in the first eleven weeks of life. This is a little faster than that documented in our preliminary note (Russell Hunter and Apley, 1966). Apley (1970) provides detailed population statistics for subadult and adult growth, and from his figures modal tissue dry-weights can be derived of 1.9 mg (at 3.5 mm shell length) after one year of life, and of 10.3 mg (at 7.5 mm shell length) after two years. Russell Hunter and Apley (1966) quote a tissue dry-weight of 81 mg (at 10.1 mm shell length), which would certainly be a snail in its third year. Since individual snails can reach shell lengths of 12.3 mm, life-span growth in real biomass terms, such as tissue dry-weight (or total organic carbon, see Apley, 1967, 1970; Apley *et al.*, 1967; Russell Hunter and Apley, 1966) can certainly encompass six orders of magnitude in 3–4 years. Comparison of the October spat size distribution of Figure 9 with the first year's growth data of Apley (1967, 1970) suggests that the smaller spat (that is, the later-hatched cohorts) are somewhat less likely to survive their first winter. There could be a minimum size

TABLE IV

Summer spring tides which corresponded to semilunar cycles of aggregation, copulation and egg-laying in populations of Melampus *at* Little Sippewisset (○ full moon, ● new moon)

1965	● May 30	○ June 14	● June 29	○ July 13
1966		○ June 3	● June 18	○ July 2
1967		● June 8	○ June 22	● July 7
1968	● May 27	○ June 10	● June 25	
1970	○ May 21	● June 4	○ June 19	● July 3

at November for survival to the following spring. If this is so, then the termination of the overall reproductive period in mid-July could have adaptive significance (*e.g.*, August spawning could not produce winter-viable spat) as well as reflecting bioenergetic depletion (see Results section I above).

Annual aspects of synchrony. Over all summers covered by our observations at Little Sippewisset (1965, 1966, 1967, 1968, 1970) the earliest natural egg-laying was on May 23, and the latest on July 13. At this latitude therefore the total reproductive period could extend through a maximum of 51 days. Whether this annual reproductive period encompasses either three or four cycles of copulation and egg-laying would seem to depend on the incidence of spring tides in that particular year, and on an interaction of such *potential times of egg-laying* with the limits set to total egg-production by the sequential bioenergetic depletion. The spring tides corresponding to cycles of copulation and egg-laying for the five years of observations are set out in Table IV. It may be significant that not all cycles of egg-laying are of equal intensity. In both "four-cycle" years, 1965 and 1970, the second and fourth cycles involved massive oviposition, with the first and third being slighter. On these bases, a predictive hypothesis might run as follows. (1) No spring tide before May 21 can support a breeding cycle (on

grounds of the latent period after day-length and temperature control). (2) A reproductive period of four breeding cycles can occur only if: (a) the first cycle corresponds to spring tides *before* June 1, and (b) at least one of the first three cycles does not involve massive egg-laying. This hypothesis would accommodate the case of 1968 as being one where the spring tides around July 10 constituted a potential egg-laying cycle, but depletion had already occurred with the third actual egg-laying cycle around June 25.

As in many other biological situations involving synchronization with seasonal changes, the environmental signals utilized by our populations of *Melampus* to control the timing of their reproductive period are not necessarily related to the selective pressures originally responsible for the evolution of that synchronization. The onset of the period is based on the sensory inputs of appropriate day-length and of temperature. The ending of reproduction must involve detection of an environmental cue, as well as the immediate effects of gonad depletion. We have no direct evidence on this, although changing day-length is a more likely factor than temperature in mid-July. Obviously, the adaptive significance of terminating reproduction in mid-July (to ensure the settlement and growth of winter-viable spat) need not be so important at lower latitudes. Earlier reports on reproduction in *Melampus* (Hausman, 1936; Holle and Dineen, 1957; Morrison, 1958) seem to have been based on isolated observations of single breeding cycles, with no appreciation of the semilunar rhythm of reproduction. Morrison (1958) briefly discussed the "problem" of an apparent progression of egg-laying dates from north to south. A progressively *later* onset of reproduction within species of marine littoral invertebrates as one moves to populations in higher latitudes is much more usual (Runnström, 1928; Spärck, 1933; Thorson, 1936, 1946; Hutchins, 1947; Jenner, 1956). Apley (1970) records three breeding cycles with semilunar periodicity in late August and September for a population of *Melampus bidentatus* at Fort Macon, North Carolina. Fort Macon is at 34° 43′ N, compared to 41° 35′ N for Little Sippewisset. The most likely explanation involves the existence of different physiological races of *Melampus* at these different latitudes. It is of interest that Sastry (1970) has postulated similar racial differences between two populations of the bay scallop, *Aequipecten irradians*, in Nantucket Sound, Massachusetts and off Beaufort, North Carolina, this being another of the "unusual" cases where the population at the higher latitude spawns earlier in the year. In the case of *Melampus*, we can now postulate that the evolution of such races may have involved the absence of selection pressures for winter-viable spat in the Fort Macon population where winter temperatures in the salt marshes rarely fall below 5° C. In addition, the best conditions for planktonic veligers off Fort Macon may occur later in the summer, since Williams and Murdoch (1966) have reported peak primary productivity during the summer (rather than spring) months for that sea area.

Discussion

Two aspects of the early life-history of *Melampus* merit further discussion: first, the significance of growth and survivorship problems resulting from the relatively small egg, including the mantle metamorphosis after settlement, and secondly the overall significance of semilunar synchrony.

Among molluscs, as among other marine invertebrates, there are cases of pairs of relatively closely related species differing markedly in their specific egg-sizes (and thus differing inversely in their numerical fecundity). The evolution of larger eggs has involved a balance of selection pressures: small eggs confer advantages in species distribution, and perhaps also in any species' capacity for genetic change; while large eggs confer advantages in survivorship resulting from suppression of free larval stages, and also (as we have suggested earlier, Russell Hunter and Apley, 1966) in reduction of the temporal extent of immature growth. As documented in this paper, in *Melampus* biomass growth in terms of organic carbon or ash-free dry-weight extends through two orders of magnitude (1×10^2) during veliger and early spat (under 675μ) life, through over three orders (actually 1.6×10^3) during the first eleven weeks of life, and through just over six orders ($> 1 \times 10^6$) in the entire life-span. In contrast, our studies show a variety of "higher" freshwater pulmonates hatching from relatively large eggs (5–40 μg organic carbon), and showing a biomass growth of only two to three orders of magnitude (about 5×10^2) during their life-span [*Physa heterostropha* (Russell Hunter and Apley, 1966); *Ferrissia rivularis* (Burky, 1971); *Lymnaea palustris* (Hunter, 1972); *Laevapex fuscus* (McMahon, 1972); and *Ancylus fluviatilus* (Russell-Hunter and Burky, in preparation)]. The eggs of most land pulmonates are proportionately even larger, and the extent of biomass growth from egg to adult still further reduced. Discussions of the evolution of larger eggs in nonmarine environments usually emphasize the "need to suppress the free larval stages." Our data on the orders of magnitude involved in real measures of growth, such as organic carbon, suggest that selection pressures to reduce the temporal extent of immature growth have also influenced the evolution of larger eggs. The "start in life" of being born large is important in environments with marked seasonal changes and, in the various species of freshwater pulmonates noted above, has made possible both strict annual and certain bivoltine patterns of life-cycle (see also Russell-Hunter, 1964, 1970).

In most molluscs, as in certain other invertebrates, there is a close inverse relationship between egg-size and numerical fecundity which reflects the maximum possible production of egg-biomass by the parent. Most benthic marine invertebrates with small eggs yielding planktonic larvae produce egg numbers in the range 10^3 to 10^5 per female per breeding season (Thorson, 1950; see also Scheltema, 1971). The numerical fecundity of the hemaphroditic *Melampus* lies neatly within this range at 33,150 eggs per snail per year. Numerical fecundities for the large-egged freshwater pulmonates listed above (all also hermaphroditic) lie in the range 8–800 eggs per snail per year. Retention of the "primitive" pattern of small eggs and high numerical fecundity in *Melampus* must reflect long-term adaptive advantages. These include the enhanced species distribution which results from a planktonic period in the life-cycle since the habitat provided by salt marshes is geographically discontinuous. Another factor which may be of long-term adaptive significance is the increased capacity for genetic change conferred by a higher numerical fecundity and a longer elapsed time between hatching and maturity (the period of effective natural selection). Obviously the probability of survival to maturity of any individual egg in a species with near-Darwinian populations (nearly constant population numbers from generation to generation) is inversely propor-

tional to the average life-time numerical fecundity. In reporting earlier molluscan studies, Russell Hunter (1957) had used an "annual ratio of selection in stable populations" (page 69) to express this relationship. If the life-time fecundity of *Melampus* is prorated at about 55,000 (applying the age-structure and survival of natural populations at Little Sippewisset to our mean laboratory fecundity of 33,150 per snail per reproductive season), then this can be contrasted with life-time fecundities in the range 8–70 for different populations of certain ancylid limpets (*Ancylus*, *Ferrissia* and *Laevapex*: Russell Hunter, 1953a; Burky, 1971; McMahon, 1972). Ancylid limpets can be regarded as among the most highly specialized of freshwater pulmonates, and the low level of fecundity is probably correlated with this. If the "primitive" pattern of reproduction in *Melampus* with high numerical fecundity confers certain long-term adaptive advantages, some of the short-term disadvantages in a more stochastic pattern of survivorship must be countered to some extent by the multiplication of separate egg-laying cycles within each reproductive period.

Again as a result of the relatively small egg and the need for active food intake and growth as a veliger, *Melampus* shows a profound trophic shift at settlement— a functional metamorphosis if not a change in form. Filter-feeding by velar cilia must be replaced within a few hours by the processes of active radular grazing which will continue throughout adult life. The more obvious metamorphosis of shell which occurs a little later (some days after settlement) in *Melampus* is one of the mantle-edge and of the secreted shell shape which it generates (see Section III, above) and this metamorphosis does *not* involve any reversal of asymmetric internal organs such as the kidney and the auricle of the heart. Throughout the embryonic, larval and spat stages of *Melampus*, the internal organs are in their adult *dextral* arrangement. Thus the terms sinistral (for the late veliger and post-larval shells) and dextral (for the shells of metamorphosed spat and of adults) were used above only in their simpler descriptive sense as regards the apparent direction of shell-coiling but *not* in the more specific sense used in gastropodan comparative anatomy as regards a mirror-image asymmetry of all the unpaired internal organs. If the terms sinistral and dextral are restricted to this morpho-logical usage for cases of entire reversal of symmetry or *situs inversus* of all organ systems (which in turn reflect mirror-reversal of the planes of spiral cleavage in the egg), then we must describe the shells of the later veliger and postlarva in *Melampus* (Figs. 3AB and 4AB) as hyperstrophic, and the shells of metamor-phosed spat and of adults (Figs. 6ABC and 7ABC) as orthostropic. An early elucidation of hyperstrophy (or those cases of apparently sinistrally coiled shells enclosing snails with dextrally arranged internal anatomy) occurs in Simroth (1896–1907; see also Pelseneer, 1891, 1892, 1906), and more recent accounts include those of Brookes Knight (1952), Cox (1960), and Fretter and Graham (1962). Metamorphosis of the mantle and shell in *Melampus* from a hyperstrophic to an orthostrophic condition (with dextrally coiled shell enclosing dextral anat-omy) is paralleled in a number of other snails with planktonic larvae (Fretter and Graham, 1962; Robertson, 1963; Robertson and Merrill, 1963). Among these are many species of those Pyramidellidae (a family now regarded as opisthobranch) which have planktonic veligers. Apparently those pyramidellids in which the planktonic veliger is suppressed do not show any heterostrophy or shell meta-

morphosis. As Fretter and Graham (1962) have discussed, the pyramidellids have many features which could be interpreted as those of a "stem group" transitional between opisthobranchs and prosobranchs. That the nature of shell metamorphosis is *Melampus* should be similar is striking, since Morton (1955a, 1955b) has pointed out the many archetypic features of nervous, excretory and reproductive anatomy in ellobiid pulmonates which appear to link the higher pulmonates through them to the same prosobranch stock (the Rissoacea-Cerithiacea group of monotocardians) from which the opisthobranchs may have evolved (see also Fretter and Graham, 1962).

No matter the significance of such attempted phyletic correlations (and extensive dialectics on the significance of torsion are involved here), and despite the complex terminology of shell-coiling, a simple explanation in adaptive-functional terms can be put forward for most, perhaps all, of the growth changes of the mantle-shell in *Melampus,* including the metamorphosis. The explanation, mechanistic and Ockhamistic, must lie in the changing needs for protective containment of the changing proportions of the head-foot and visceral mass. The genetic controls, which create the growth gradients of the shell aperture by generating the differential rates of cell division in the cells of the mantle edge, have been adapted to switch appropriately to best enclose the changing asymmetries of the internal parts. Within the egg-shell, even after torsion, growth of the mantle-edge is bilaterally symmetric and, if continued, would generate a planospiral shell. During planktonic life, the mantle of the veliger produces a shell with a rapidly enlarging aperture and relatively disjunct or "open" turbinate coiling. For this period, shell growth shows some allometry. As already noted (Observations, Section III), both these shell features and the retention of an operculum can be explained by the need to accommodate the large velar lobes. After settlement, loss of the velum is accompanied by isometric and slower enlargement of the shell aperture in the postlarva. If this growth continued, we should have a hyperstrophic shell through the adult, but *it does not* and we have that profound metamorphosis of the mantle which leads to the generation of the orthostrophic (true dextral) coiling of spat and adult. With moderately turbinate shell growth, adult hyperstrophy would bring the spire anteriorly (or at least anteriolaterally on the left side) and, despite torsion, would result in similar disadvantages (in the mechanics of locomotion and of sanitation) to those hypothesized for exogastric as opposed to endogastric coiling (Pelseneer, 1906; Naef, 1911; Yonge, 1947; Ghiselin, 1966). It seems highly significant that mantle-shell metamorphosis in *Melampus* occurs only a few days after settlement and the shift from a swimming to a crawling habit. As now seems to hold for certain peculiarities earlier claimed in the shell growth of ancylid limpets (Russell Hunter, 1953a; Russell-Hunter and Nickerson, in preparation, on biometrics of *Ferrissia*), the simplest adaptive interpretation in terms first of the necessary containment of head-foot with visceral mass, and secondly of locomotory efficiency, may best account for all the shifts of shell-mantle growth in *Melampus.*

The unique combination of primitive and of specialized features, which characterize different aspects of the physiology of *Melampus,* creates the need for the strict synchronization of such processes as egg-laying, hatching and settlement. Although amphibious snails, in their respiration they are relatively specialized, breathing air through pneumostome and lung as in the true land snails. Adult

specimens of *Melampus* can be drowned by prolonged submergence. It appears that *Melampus* is unable to use an exposed gas bubble as a "physical gill" for sustained diving, as can be done in certain pulmonate genera (including *Lymnaea, Physa* and *Planorbarius*) which have become "readapted" for aquatic life in fresh waters (see Russell Hunter, 1953b; Henderson, 1963; Russell Hunter, 1964). Given this inability to live submerged, and the general topography of salt marshes, it is clear why adults of *Melampus* do *not* migrate down to lower zones of the littoral for reproduction as do some sympatric arthropods. On the other hand, the relatively primitive pattern of reproduction in *Melampus* involves the spawning of large numbers of small eggs which yield planktonic larvae on hatching.
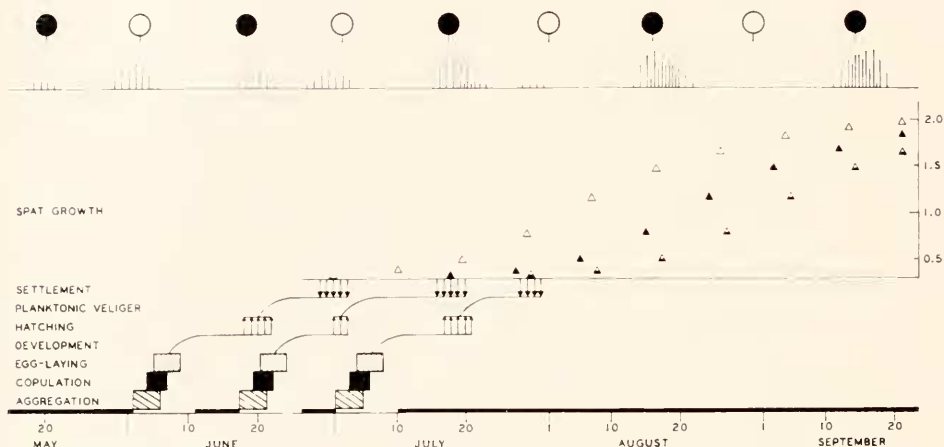

FIGURE 10. Semilunar periodicity in reproduction and early life-cycle of *Melampus* at Little Sippewisset. The diagram is based on the actual timings and observations of the "three-cycle" reproductive period of summer 1966, except for the spat growth which is interpolated from the more extensive spat data of 1970. At the top of the figure lunar phases are shown conventionally, with below them a record of those high tides which exceeded a vertical height of 4.4 feet (the height of the top of the *Melampus* zone in Fig. 8). At the bottom of the figure, the thicker portions of the base-line indicate times when the population of *Melampus* was dispersed. Three cycles of aggregation, copulation and egg-laying occur at semilunar intervals. Hatching and settlement also show semilunar synchronization. Three kinds of triangles are used to distinguish successive modal sizes for the three cohorts of spat which result from the three cycles of egg-laying in this reproductive period, and the vertical scale at the right indicates the maximum shell dimension of those spat in millimeters. For further explanation and discussion, see text.

As specialized lung-snails, populations of *Melampus* live in the upper 12% of the intertidal zone. Since they retain the reproductive pattern of primitive marine snails, they can spawn only during the 2.3% to 4% of each semilunar tidal cycle when their habitat is bathed by seawater. Hatching of planktonic veligers and resettlement must also be similarly synchronized. The acuity of these problems of temporal "fitting" in *Melampus*, as compared with other intertidal animals, results from those two divergent sets of physiological features. The considerable evolutionary interest of the ecology and physiology of ellobiid snails like *Melampus* stems

from this combination of the primitive and the specialized. Contemporary ellobiids possibly live in similar habitats to those of the Jurassic ancestors of the successful land-snails of today, and undoubtedly they face similar physiological problems. The retention of archetypic reproduction in *Melampus* over this long evolutionary period implies not only successful, but also adaptable, methods of seasonal and semilunar synchronization. The possible occurrence of physiological races of *Melampus* adapted to different latitudes is significant in relation to this apparent evolutionary conservatism. Even more striking in this regard is the contemporary existence in the controls of semilunar synchronization both of obligate and of facultative processes—a mixture which undoubtedly is of adaptive significance in conferring a flexibility of resynchronization and thus a long-term capacity to adjust to varying tidal circumstances.

The first necessary synchronization, that of aggregation, copulation and egg-laying with the days of spring tides, is an obligate process. The appropriate behavioral shifts and the internal changes in the reproductive tract preparatory to copulation and egg-laying occur in laboratory stocks at times of full and of new moon, even when these stocks have not experienced the rhythms of tidal submergence for ten semilunar cycles. It is not unlikely that the day-length and other controls of the onset of the annual reproductive period are "paced" to some extent by the same "biological clock," or perception of more subtle semilunar geophysical changes, as dictates the rhythm of copulation and egg-laying. Whatever the mechanism, egg-laying occurs with a semilunar synchrony despite the absence of tidal bathing.

The second synchronization, that of hatching, is different. Conceivably, it too could be obligate and depend on a rigorous developmental timetable following synchronous fertilization, egg-laying and first cleavage, although the necessary temperature independence is somewhat unlikely. In fact, as reported above, the synchronous hatching process is facultative and depends on the incidence of tidal submergence. As confirmed by our laboratory experiments, hatching of *Melampus* in the field must usually be brought about from egg-masses of age over 10 days (and under 21 days) by a sequence of about four tidal floodings. The adaptational significance of the innate flexibility in the time of hatching is that, by this extension of survival, it can provide for the successful overlap of cohorts from different semilunar cycles of egg-laying. On the other hand, although hatching is a facultative process, a resynchronization of the early life-history in *Melampus* is achieved by the dependence on a sequence of floodings (occurring with a semilunar periodicity in the field), and this resynchronization is undoubtedly of great significance to the future synchronous resettlement of veligers into the appropriate salt-marsh habitat at a later cycle of spring high tides.

Settlement is an important problem for all intertidal benthic invertebrates with planktonic larval stages. In the majority of such forms there are physiological and behavioral adaptations which effectively increase the chances of resettlement into the appropriate zonal habitat. Aspects of the interaction of temporal controls and the immediate environmental stimuli on settlement of planktonic larvae have been ably summarized by Wilson (1948, 1952), who also provides one of the best expositions of the important ability of *some* larvae to delay settlement long after they have reached an appropriate stage of development, if in the meantime they have

not encountered the specific environmental conditions appropriate to their settlement. Recently Scheltema (1971) has discussed the importance of zoögeographical distribution of this capacity for prolongation of larval life in some prosobranch gastropods. Veligers of *Melampus* seem to have some capacity to delay settlement —at least for a few days. The problems of resettlement into the appropriate intertidal zone are especially acute for animals of the supralittoral fringe like *Melampus*, particularly when there can be only a limited possibility of upward migration by spat after settlement. We have no evidence of any species-specific chemoreception (either repellant, see Wilson, 1952; or attractant, see Knight-Jones, 1953) being important in the settlement of *Melampus*. Although a few larvae settle just *below* the adult *Melampus* zone, the great bulk of settlement occurs into that exact zone. Thus it occurs in the field only at spring high tides. Although some aspects of this remain obscure, settlement as a process undoubtedly involves only veligers of a suitable age and size, and may involve a definite level of local mechanical stimuli for completion.

Semilunar synchronization of the events of reproduction and of early life-cycle is clearly necessary in contemporary populations of *Melampus*. A long evolutionary history of the capacity for this rhythm is suggested by the retention of an archetypic pattern of reproduction and larval life. In turn, this could have been made possible by the flexibility of resynchronization conferred by the use of a combination of obligate and facultative processes to achieve the required synchrony at each stage.

Summary

1. The salt-marsh pulmonate snail, *Melampus bidentatus*, is placed in the Ellobiidae which family encompasses the most primitive of living Pulmonata and is regarded as not far removed from the ancestral stem-group of both modern land snails and freshwater pulmonates. Inhabiting the higher levels of salt marshes, *Melampus* is "amphibious": although an air-breather with a gill-less vascularized mantle-cavity functioning as a lung, it retains an archetypic pattern of reproduction with small eggs and a free-swimming veliger larva.

2. Field and laboratory studies over several years (based on natural populations at Little Sippewisset, Cape Cod, Massachusetts) have shown that egg-laying, hatching, and larval settlement are each confined to cycles of about four days in phase with the spring high tides. Adaptively such semilunar synchronies ensure that these processes occur only during the 2.3% to 4% of each month when the *Melampus* habitat in the upper 12% of the intertidal zone is bathed by seawater.

3. The annual reproductive period extends from late May or early June through early July, with either three or four cycles of egg-laying occurring at two-week intervals in phase with the tides of new and of full moon. Synchrony of egg-laying (and of the patterned aggregation and copulation which precede it) is obligate.

Stocks of *Melampus* brought into the laboratory in spring will maintain the same semilunar rhythm of reproductive behavior during the summer period.

4. Eggs are small (about 109 ng organic carbon) and are laid in gelatinous egg-masses averaging 850 eggs. Mean numerical fecundity is 33,150 eggs per snail per year. For most freshwater pulmonates fecundity would lie in the range 8–800 eggs per snail per year. At 18° C, development to a well-differentiated and active veliger within the egg-shell takes 11 days.

5. Hatching shows semilunar synchrony in the field: enormous numbers of newly hatched veligers can be collected on the flood of appropriate spring tides. A series of experiments with laboratory-laid egg-masses showed that eclosion normally occurs in response to a sequence of about 4 tidal floodings in under 50 hours. Hatching can occur from egg-masses from 10 to 24 days after laying. Being facultative, the process allows better survival and overlap of cohorts but also re-establishes the synchronization with spring tides.

6. Veligers feed actively and grow from shell length 127 μ to 280 μ during their time in the plankton, deduced to be 14 ± 2 days. The bulk of the settlement is into the exact vertical zone occupied by adult *Melampus*.

7. A period as a crawling, radula-feeding postlarva (after loss of velar lobes and operculum) is followed by an abrupt metamorphosis of the mantle and shell. Postmetamorphic spat grow rapidly. In terms of organic carbon or ash-free dry weight, growth extends through two orders of magnitude during veliger and early spat life, through more than three during the first eleven weeks, and six in the entire 3–4 year life-span. In contrast, similar biomass growth measures in freshwater pulmonates involve only two to three orders in their life-span.

8. In *Melampus,* the shells of late veligers and of post-larvae show sinistral coiling, and those of metamorphosed spat and of adults dextral coiling. There is a metamorphosis of mantle and shell alone; throughout development, larval and spat stages, the internal organs are in their adult dextral arrangement. Such a metamorphosis from a hyperstrophic shell condition to an orthostrophic one is known to occur in the ectocommensal opisthobranch family Pyramidellidae and in certain other snails with planktonic larvae. The present study provides the first description of the succession of shell stages and metamorphosis for any pulmonate.

9. In conclusion, the small eggs, the mantle-shell metamorphosis, and the semilunar synchrony are discussed in their evolutionary setting. "Primitive" reproduction with small eggs, as retained in *Melampus,* confers advantages in dispersal and genetic potential. Evolution of larger eggs, as in the freshwater pulmonates, may have involved selection pressures to reduce the temporal extent of immature growth in seasonally variable environments. Mantle-shell changes in *Melampus,* including the metamorphosis, can be interpreted simply in terms of the changing needs for protective containment at different stages in the life-cycle. Semilunar synchrony of reproductive and of larval stages has evolved in response to the concursion of specialized aerial respiration and the primitive pattern of spawning large numbers of small eggs. The combination of both obligate and facultative processes in producing these synchronies is thought to be significant in relation to the long evolutionary history which can be hypothesized for these semilunar rhythms.

LITERATURE CITED

APLEY, M. L., 1967. Field and experimental studies on pattern and control of reproduction in *Melampus bidentatus* (Say). *Ph.D. thesis, Syracuse University,* 154 pp.

APLEY, M. L., 1970. Field studies on life history, gonadal cycle and reproductive periodicity in *Melampus bidentatus* (Pulmonata: Ellobiidae). *Malacologia,* **10**: 381–397.

APLEY, M. L., W. D. RUSSELL-HUNTER AND R. J. AVOLIZI, 1967. Annual reproductive turn-over in the salt-marsh pulmonate snail, *Melampus bidentatus. Biol. Bull.,* **133**: 455–456.

BLUM, J. L., 1968. Salt marsh spartinas and associated algae. *Ecol. Monogr.,* **38**: 199–221.

BONDESEN, P., 1950. A comparative morphological-biological analysis of the egg capsules of freshwater pulmonate gastropods. *Natura Jutlandica,* **3**: 1–208.

BOSS, K. J., 1971. Critical estimate of the number of recent Mollusca. *Occas. Pap. Mollusks Mus. Comp. Zool. Harvard Univ.,* **3**: 81–135.

BURKY, A. J., 1971. Biomass turnover, respiration, and interpopulation variation in the stream limpet *Ferrissia rivularis* (Say). *Ecol. Monogr.,* **41**: 235–251.

CHAPMAN, V. J., 1940. Studies in salt marsh ecology sections VI and VII. Comparison with marshes on the east coast of North America. *J. Ecol.,* **28**: 118–152.

COX, L. R., 1955. Thoughts on the classification of the Gastropoda. *Proc. Malacol. Soc. London,* **33**: 239–261.

DAVIS, C. C., 1964. A study of the hatching process in aquatic invertebrates VII and VIII. *Hydrobiologia,* **23**: 253–266.

DAVIS, C. C., 1967. Emergence of veliger larvae from eggs in gelatinous masses laid by some Jamaican marine gastropods. *Malacologia,* **5**: 299–309.

DAVIS, C. C., 1968. Mechanisms of hatching in aquatic invertebrate eggs. *Oceanogr. Mar. Biol. Annu. Rev.,* **6**: 325–376.

FISCHER, P., AND H. CROSSE, 1900. Études sur les mollusques terrestres et fluviatiles du Mexique et du Guatemala. Part 7 in Henri Milne Edwards, Ed., *Recherches zoologiques pour servir a l'historic de la fauna de l'Amérique Centrale et du Mexique.* Imprimerie Imperial, Paris.

FRETTER, V., 1967. The prosobranch veliger. *Proc. Malacol. Soc. London,* **37**: 357–366.

FRETTER, V., AND A. GRAHAM, 1962. *British Prosobranch Molluscs, their functional anatomy and ecology.* Ray Society, London, 755 pp.

GARSTANG, W., 1928. The origin and evolution of larval forms. *Rep. Brit. Ass. Advan. Sci. Glasgow,* **1928**: 77–98.

GHISELIN, M. T., 1966. The adaptive significance of gastropod torsion. *Evolution,* **20**: 337–348.

HARRY, H. W., 1951. Growth changes in the shell of *Pythia scarabaeus* (Linné). *Proc. Calif. Zool. Club,* **2**: 7–14.

HAUSMAN, S. A., 1936. Food and feeding activities of the salt marsh snail (*Melampus bidentatus*). *Anat. Rec.,* **67**: 127.

HENDERSON, A. E., 1963. On the underwater weights of freshwater snails. *Z. Vergl. Physiol.* **46**: 467–490.

HOLLE, P. A., AND C. F. DINEEN, 1957. Life history of the salt-marsh snail, *Melampus bidentatus* Say. *Nautilus,* **70**: 90–95.

HUNTER, R. D., 1972. Energy budgets and physiological variation in natural populations of the freshwater pulmonate, *Lymnaea palustris. Ph.D. thesis, Syracuse University,* 110 pp.

HUTCHINS, L. W., 1947. The bases for temperature zonation in geographical distribution. *Ecol. Monogr.,* **17**: 325–335.

HUXLEY, J. S., 1932. *Problems of Relative Growth.* Methuen and Co., London, 265 pp.

JENNER, C. E., 1956. The timing of reproductive cessation in geographically separated populations of *Nassarius obsoletus. Biol. Bull.,* **111**: 292.

KNIGHT, J. BROOKES, 1952. Primitive fossil gastropods and their bearing on gastropod classification. *Smithsonian Misc. Collect.,* **117**(13): 1–56.

KNIGHT-JONES, E. W., 1953. Laboratory experiments on gregariousness during setting in *Balanus balanoides* and other barnacles. *J. Exp. Biol.,* **30**: 584–598.

Mapstone, G. M., 1970. Feeding activities of veligers of *Nassarius reticulatus* and *Crepidula fornicata* and the use of artificial foods in maintaining cultures of these larvae. *Helgoländer Wiss. Meersunters.,* **20** : 565–575.

McMahon, R. F., 1972. Interpopulation variation and respiratory acclimation in the bio-energetics of *Laevapex fuscus. Ph.D. thesis, Syracuse University,* 164 pp.

Morrison, J. P. E., 1958. The primitive [life] history of some salt marsh snails. *Amer. Malacol. Union Annu. Rep. Bull.,* **11** : 25–26.

Morton, J. E., 1955a. The functional morphology of the British Ellobiidae (Gastropoda Pulmonata) with special reference to the digestive and reproductive systems. *Phil. Trans. Roy. Soc. London, Series* **B239**: 89–160.

Morton, J. E., 1955b. The evolution of the Ellobiidae with a discussion on the origin of the Pulmonata. *Proc. Zool. Soc. London,* **125**: 127–168.

Naef, A., 1911. Studien zur generellen Morphologie der Mollusken. 1. Tiel: Uber Torsion und Asymmetrie der Gastropoden. *Ergebnisse und Fortschritte der Zoologie,* **3** : 73–164.

Pelseneer, P., 1891. Sur la dextrorsité de certains Gastropodes dits "sénestres" (*Lanistes, Peraclis, Limacina,* larves des Cymbullidae. *C. R. Séances Acad. Sci. Paris,* **112**(1) : 1015–1017.

Pelseneer, P., 1892. A propos de l'asymétrie des Mollusques univalves. *J. Conchyliologie,* **40**: 229–233.

Pelseener, P., 1901. Études sur des Gastéropodes Pulmonés. *Mem. Acad. Roy. Belg. Cl. Sci.,* **54**: 1–76.

Pelseener, P., 1906. Mollusca. Part V in E. Ray Lankester, Ed., *A Treatise on Zoology.* Adam and Charles Black, London, 355 pp.

Pilkington, M. C., and V. Fretter, 1970. Some factors affecting the growth of prosobranch veligers. *Helgoländer. Wiss. Meersunters.,* **20** : 576–593.

Redfield, A. C., 1972. Development of a New England saltmarsh. *Ecol. Monogr.,* **42** : 201–237.

Robertson, R., 1963. The hyperstrophic larval shells of the Architectonicidae. *Amer. Malacol. Union Annu. Rep. Bull.,* **1963** : 11–12.

Robertson, R., and A. S. Merrill, 1963. Abnormal dextral hyperstrophy of post-larval *Heliacus* (Gastropoda : Architectonicidae). *Veliger,* **6** : 76–79.

Runnström, S., 1928. Über die Thermopathie der Fortplanzung und Entwicklung mariner Tiere in Beziehung zu ihrer geographischen Verbreitung. *Bergens Museums Arbok,* **1927**: 1–67.

Russell Hunter, W., 1953a. On the growth of the freshwater limpet, *Ancylus fluviatilis* Müller. *Proc. Zool. Soc. London,* **123** : 623–636.

Russell Hunter, W., 1953b. The condition of the mantle cavity in two pulmonate snails living in Loch Lomond. *Proc. Roy. Soc. Edinburgh,* **B65** : 143–165.

Russell Hunter, W., 1957. Studies on freshwater snails at Loch Lomond. *Glasgow Univ. Publ., Stud. Loch Lomond,* **1** : 56–95.

Russell Hunter, W., 1964. Physiological aspects of ecology in nonmarine molluscs. Pages 83–126 in K. M. Wilbur and C. M. Yonge, Eds., *Physiology of Mollusca,* Volume 1. Academic Press, New York and London.

Russell-Hunter, W. D., 1968. *A Biology of Lower Invertebrates.* The Macmillan Company, New York, 181 pp.

Russell-Hunter, W. D., 1970. *Aquatic Productivity.* The Macmillan Company, New York, 306 pp.

Russell Hunter, W., and M. L. Apley, 1966. Quantitative aspects of early life-history in the salt-marsh pulmonate snail, *Melampus bidentatus,* and their evolutionary significance. *Biol. Bull.,* **131** : 392–393

Russell-Hunter, W. D., M. L. Apley and R. D. Hunter, 1970. Semilunar and other factors influencing hatching from egg-masses of *Melampus bidentatus* in the field and in the laboratory. *Biol. Bull.,* **139** : 434.

Russell-Hunter, W. D., R. T. Meadows, M. L. Apley and A. J. Burky, 1968. On the use of a 'wet-oxidation' method for estimates of total organic carbon in mollusc growth studies. *Proc. Malacol. Soc. London,* **38** : 1–11.

SASTRY, A. N., 1970. Reproductive physiological variation in latitudinally separated populations of the bay scallop, *Aequipecten irradians* Lamarck. *Biol. Bull.*, **138**: 56–65.

SCHELTEMA, R. S., 1971. Larval dispersal as a means of genetic exchange between geographically separated populations of shallow-water benthic marine gastropods. *Biol. Bull.*, **140**: 284–322.

SIMROTH, H., 1896–1907. Gastropoda Prosobranchia. [Sections making up part 3]. Pages 1–1056 in Bronn, H. G., Ed., *Klassen und Ordnungen des Tierreichs*, 1892–1940. Friedlander und Sohn (and) Akademische Verlagsgesellschaft, Leipzig.

SOUTHWARD, A. J., 1958. The zonation of plants and animals of rocky sea shores. *Biol. Rev.*, **33**: 137–177.

SPÄRCK, R., 1933. Contributions to the animal ecology of the Franz Joseph Fjord and adjacent East Greenland waters, I–II. *Medd. Grønland*, **100(Nr1)**: 1–38.

STEPHENSON, T. A., AND A. STEPHENSON, 1948. The universal features of zonation between tide-marks on rocky coasts. *J. Ecol.*, **37**: 289–305.

STRATHMANN, R. R., T. L. JAHN AND J. R. C. FONSECA, 1972. Suspension feeding by marine invertebrate larvae: clearance of particles by ciliated bands of a rotifer, pluteus and trochophore. *Biol. Bull.*, **142**: 505–519.

THIELE, J., 1931. *Handbuch der Systematischen Weichtierkunde*. Fischer, Jena, 778 pp.

THORSON, G., 1936. The larval development, growth and metabolism of arctic marine bottom invertebrates compared with those of other seas. *Medd. Grønland*, **100(Nr6)**: 1–155.

THORSON, G., 1946. Reproduction and larval development of Danish marine bottom invertebrates, with special reference to the planktonic larvae in the sound (Øresund). *Medd. Komm. Dan. Fisk. Havendersøg. Copenhagen Series-Plankton*, **4**: 1–523.

THORSON, G., 1950. Reproductive and larval ecology of marine bottom invertebrates. *Biol. Rev.*, **25**: 1–45.

WILBUR, K. M., AND G. OWEN, 1964. Growth. Pages 211–242 in K. M. Wilbur and C. M. Yonge, Eds., *Physiology of Mollusca*, Volume 1. Academic Press, New York and London.

WILLIAMS, R. B., AND M. B. MURDOCH, 1966. Phytoplankton production and chlorophyll concentration in the Beaufort Channel, North Carolina. *Limnol. Oceanogr.*, **11**: 73–82.

WILSON, D. P., 1948. The relation of the substratum to the metamorphosis of *Ophelia* larvae. *J. Mar. Biol. Ass. U. K.*, **27**: 723–760.

WILSON, D. P., 1952. The influence of the nature of the substratum on the metamorphosis of the larvae of marine animals, especially the larvae of *Ophelia bicornis* Savigny. *Ann. Inst. Oceanogr.*, **27**: 49–156.

YONGE, C. M., 1947. The pallial organs in the aspidobranch Gastropoda and their evolution throughout the Mollusca. *Phil. Trans. Roy. Soc. London*, **B232**: 443–518.